



UNIVERSITÀ DI PISA

Facoltà di medicina e chirurgia

Corso di dottorato in fisiopatologia medica e farmacologia

Anno 2004-2006

The effects of physical activity, antioxidants and dietary intake on arterial stiffness and endothelial function in hypertensive and normotensive subjects

Yvonne Plantinga

Promoter

Chiar.mo Prof. Stefano Taddei

Tutor

Lorenzo Ghiadoni, MD, PhD

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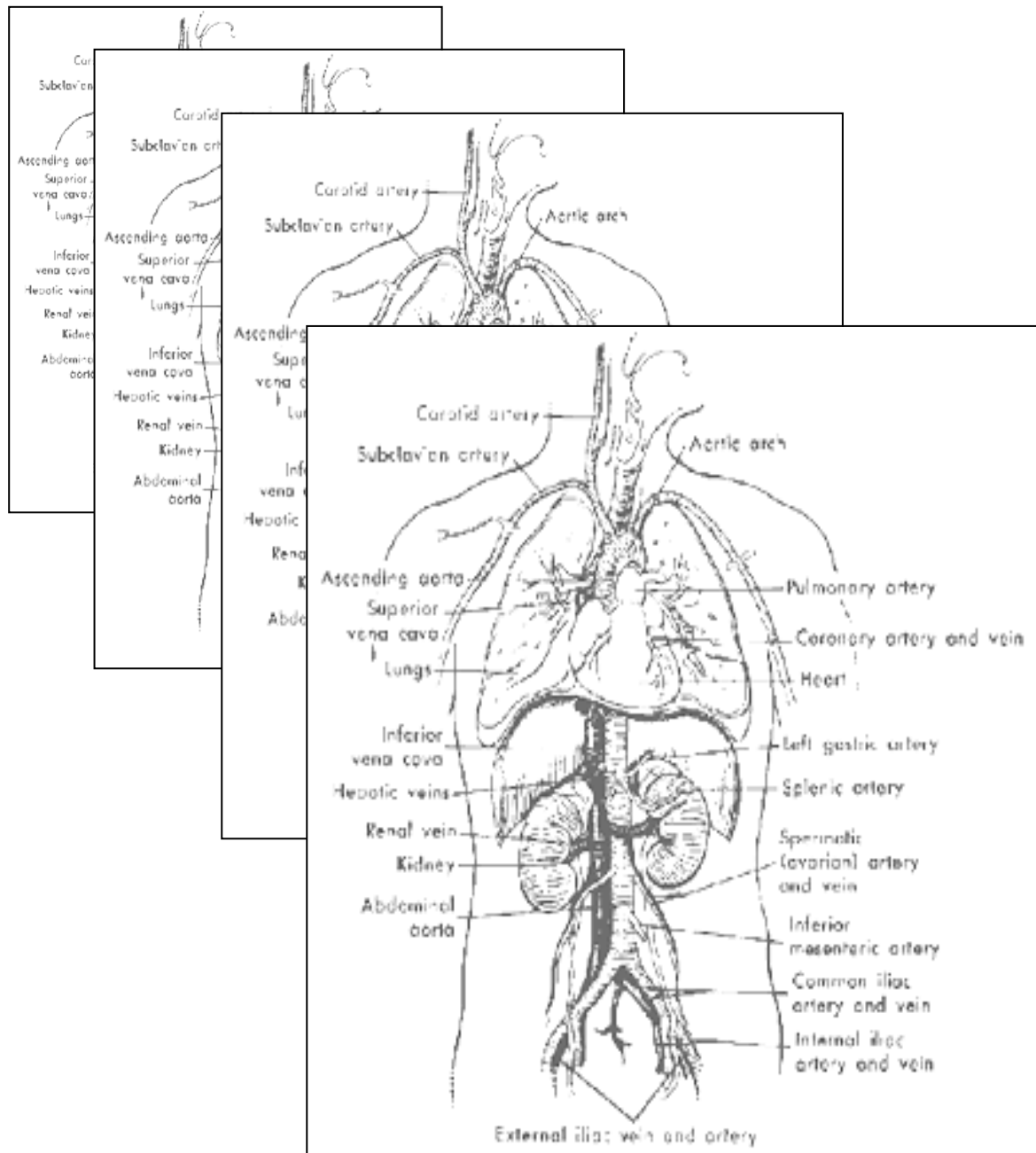
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Financial support from Sphygmocor, VEGA S.p.A. Italy, for the publication of this thesis is gratefully acknowledged.

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Summary

Essential hypertension is associated with early vascular alterations, such as endothelial dysfunction, increased arterial stiffness and wave reflection, which are considered independent predictors of cardiovascular events. Since the clustering of risk factors, characterizing the metabolic syndrome, is associated with increased cardiovascular morbidity and mortality in hypertensive patients, the first aim of this study was to evaluate the effect of the presence of the metabolic syndrome on endothelial dysfunction, arterial stiffness and wave reflection in a population of untreated essential hypertensive patients.

The most important non-pharmacological interventions in the prevention of cardiovascular disease are represented by physical activity and dietary changes. These interventions might have favorable effects on endothelial dysfunction, arterial stiffness and wave reflection. Therefore, the second aim of the study was to evaluate the effects of physical activity, dietary intake and supplementation of antioxidants on these vascular parameters in patients with essential hypertension.

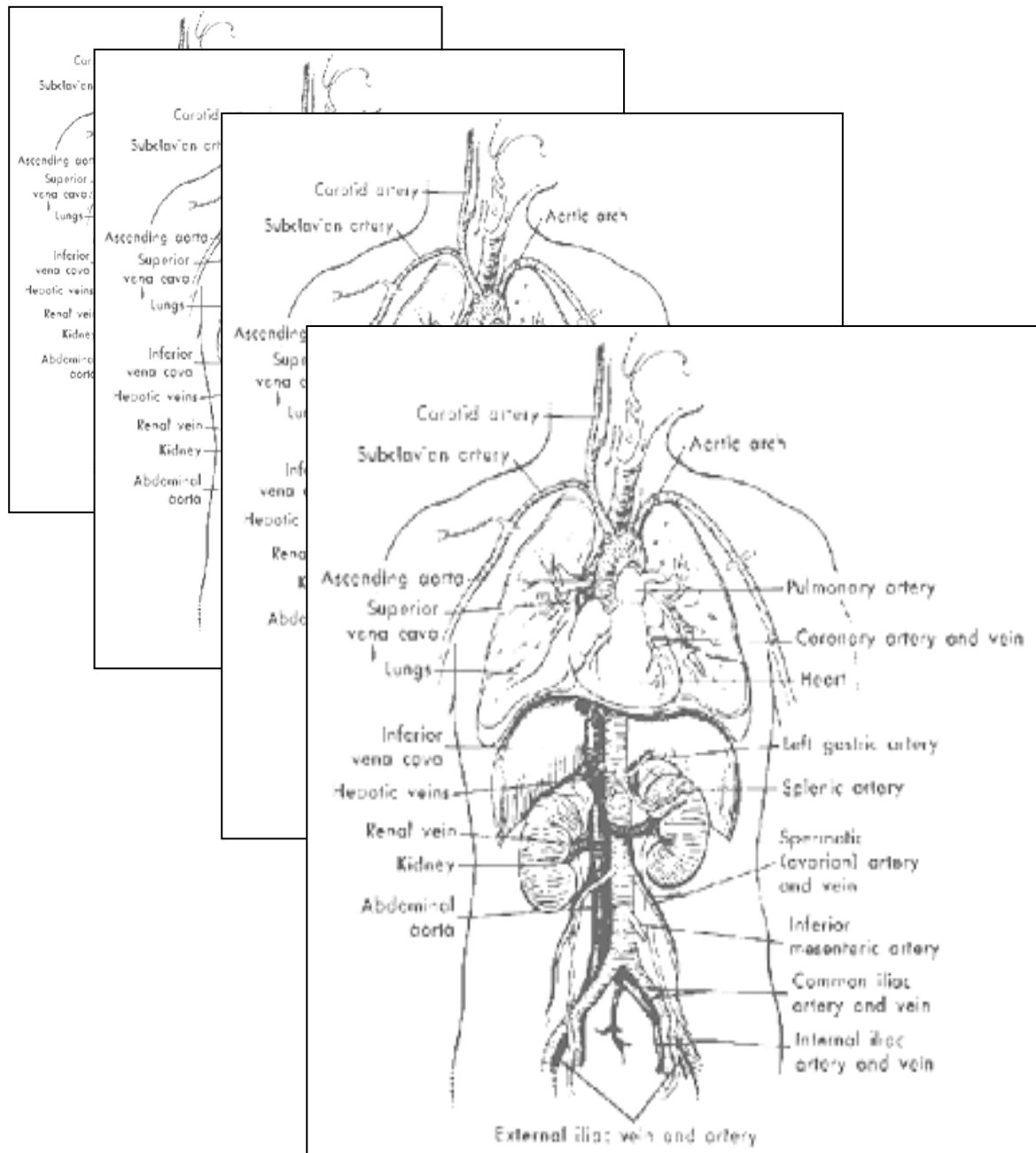
Cross sectional designs and a randomized, double blind, placebo controlled, crossover study design were used to address these research questions. Endothelium-dependent response was assessed as flow mediated dilation of the brachial artery. Arterial stiffness was assessed as central pulse wave velocity and augmentation index. The levels of habitual physical activity and dietary intake were assessed by validated questionnaires. Finally, plasma markers of oxidative stress and antioxidant status were measured.

We demonstrated that the presence of the metabolic syndrome does not account for a further deterioration of vascular structural and functional parameters in our population of untreated hypertensive patients. Moreover, blood pressure was the most important component of the metabolic syndrome in defining conduit artery endothelial function and augmentation index in normotensive subjects, suggesting the primary role of high blood pressure in inducing early vascular alterations.

Habitual physical activity was associated with a lower augmentation index in normotensive subjects and, to a lesser extent in untreated hypertensive patients. Thus, untreated essential hypertension seems to partially level out the beneficial impact of habitual physical activity on wave reflection. In a sample of healthy older individuals who performed regular and sustained endurance exercise training, we showed higher conduit artery endothelial function as compared to sedentary peers. This result was associated with preserved plasma antioxidant

defenses, suggesting a protective role of regular intensive physical exercise on vascular oxidative stress. Although our cross sectional study showed that the levels of habitual intake of the antioxidant vitamins C and E in regular food were not associated with augmentation index and endothelial function in hypertensive patients and normotensive subjects, we demonstrated a beneficial effect of supplementation with combined vitamin C and E on endothelium-dependent vasodilation and arterial stiffness in a group of untreated hypertensive patients. This effect was associated with changes in plasma markers of oxidative stress. These results underline the necessity of adequate doses of combined antioxidants to exert beneficial effect on vascular parameters.

Finally, results obtained in our cohort of hypertensive patients and control subjects suggest that the measurements of conduit artery endothelial function, central and peripheral arterial stiffness assess different entities of structure and function of the vascular tree. Therefore, in the risk assessment and in studies evaluating vascular function we recommend measuring both endothelial function and arterial stiffness.



Chapter 1

INTRODUCTION

Cardiovascular diseases (CVD) are the leading cause of mortality in the developed regions ^{1, 2} of the world. In both developing and developed regions, high blood pressure is one of the major causes of disease burden ^{1, 2}. The age- and sex-adjusted prevalence of hypertension is 28% in the North American countries and 44% in the European countries at the 140/90 mm Hg threshold ³. Hypertension prevalence correlates with cardiovascular (CV) mortality and morbidity, in both male and female and in all age ranges ⁴.

The last European guidelines on management of hypertension underline the importance of the evaluation of the total cardiovascular risk in hypertensive patients. Historically, therapeutic intervention thresholds for the treatment of cardiovascular risk factors such as blood pressure, blood cholesterol and blood sugar have been based on variably arbitrary cutpoints of the individual risk factors. Because risk factors cluster in individuals and there is a graded association between each risk factor and overall cardiovascular risk ^{5, 6}, a stratification for total cardiovascular risk in hypertensive patients (ESH-ESC guidelines) is based on the presence of multiple risk factors, including abdominal obesity, in order to give specific attention to associated conditions such as for instance the metabolic syndrome ⁷.

Figure 1 - Stratification of risk to quantify prognosis according to ESH-ESC guidelines

Other risk factors and disease history	Blood pressure (mmHg)				
	Normal SBP 120–129 or DBP 80–84	High normal SBP 130–139 or DBP 85–89	Grade 1 SBP 140–159 or DBP 90–99	Grade 2 SBP 160–179 or DBP 100–109	Grade 3 SBP ≥ 180 or DBP ≥ 110
No other risk factors	Average risk	Average risk	Low added risk	Moderate added risk	High added risk
1–2 risk factors	Low added risk	Low added risk	Moderate added risk	Moderate added risk	Very high added risk
3 or more risk factors or TOD or diabetes	Moderate added risk	High added risk	High added risk	High added risk	Very high added risk
ACC	High added risk	Very high added risk	Very high added risk	Very high added risk	Very high added risk

ACC, associated clinical conditions; TOD, target organ damage; SBP, systolic blood pressure; DBP, diastolic blood pressure.

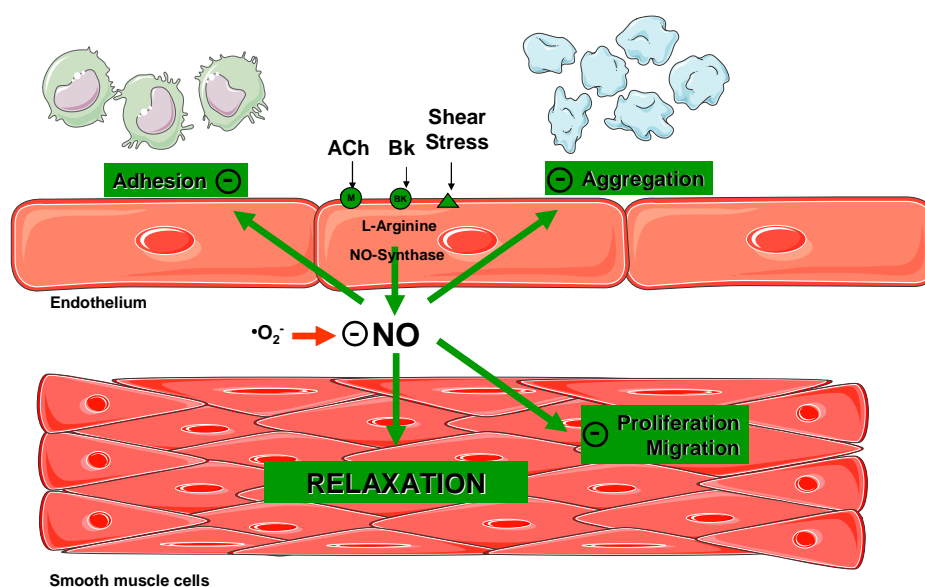
The metabolic syndrome is a metabolic disorder consisting of a clustering of cardiovascular risk factors, including hypertension, abdominal obesity, dyslipidemia (low HDL cholesterol), hypertriglyceridemia and hyperglycemia ⁸, carrying an increased risk for cardiovascular disease. In hypertensive patients the presence of the metabolic syndrome is associated with a poor cardiovascular outcome ⁹. The stratification of CV risk factors is furthermore based on

the presence of target organ damage (and associated clinical conditions) ¹⁰. The target organ damage includes left ventricular hypertrophy (LVH), increased intima-media thickening or plaques of carotid arteries, slight decrease in renal function and microalbuminuria. Finally, the actual ESH-ESC guidelines suggest that early vascular alterations, namely endothelial dysfunction and increased arterial stiffness and wave reflection might represent intermediate end-points for evaluation of the cardiovascular risk in hypertensive patients.

Endothelial Function

The endothelium is an autocrine-paracrine organ, which controls vascular tone and vascular structure. The endothelium produces multiple relaxing factors, with nitric oxide (NO) as the most important ¹¹. NO is produced and released under the influence of endothelial agonists, such as acetylcholine and bradykinin, acting on specific endothelial receptors, and by mechanical forces, such as shear stress (figure 2). Healthy endothelium provides vascular tone and inhibits smooth muscle cell growth, blood plate aggregation and the adhesion of white blood cells ¹¹ (figure 2). By definition endothelial dysfunction is a functional and reversible alteration of endothelial cells, resulting from impairment in NO availability ¹² and is nowadays considered an early and major promoter for atherosclerosis and thrombosis.

Figure 2 - Protective effect of the endothelium on vascular wall. NO: nitric oxide; $\bullet\text{O}_2^-$: oxygen free radical; Ach: acetylcholine; BK: bradykinin



In humans, endothelial function can be evaluated in different vascular districts, including the coronary and peripheral circulation. Intra-arterial infusion of endothelial agonists, such as acetylcholine, and local increase in shear stress has been used to study endothelium dependent vasodilation.

However, the most widely used non-invasive technique to measure endothelial function is flow-mediated dilation (FMD) of the brachial artery. It assesses with high-resolution ultrasound the endothelium dependent change in artery diameter induced by reactive hyperemia and can be reported as the change in artery diameter after a period of induced ischemia compared to baseline artery diameter ¹³.

Multiple studies already indicate that endothelial dysfunction is a characteristic of subjects with essential hypertension. However, endothelial dysfunction is not specific to essential hypertension, but rather results from cardiovascular risk factors, such as smoking, hypercholesterolemia, diabetes and aging ¹². Furthermore, the metabolic syndrome has been associated with a high risk of cardiovascular disease in hypertensive patients ⁹ and it has been shown that the forearm response to acetylcholine is blunted in insulin resistant hypertensive patients in the presence of the metabolic syndrome as compared to hypertensive patients without the metabolic syndrome ¹⁴. However, in clinically healthy older men, the metabolic syndrome is not associated with impaired endothelial function, measured as flow mediated dilation of the brachial artery ¹⁵.

CV risk factors are also accompanied by the presence of increased oxidative stress, defined as an excessive amount of oxidative substances, relative to endogenous antioxidant capacity which can inactivate NO ^{16, 17}. Indeed, endothelium-dependent vasodilation to acetylcholine has shown to be significantly blunted in the forearm microcirculation of hypertensive patients ¹⁶ and this alteration is caused by increased vascular oxidative stress, which reduces NO availability ¹⁶.

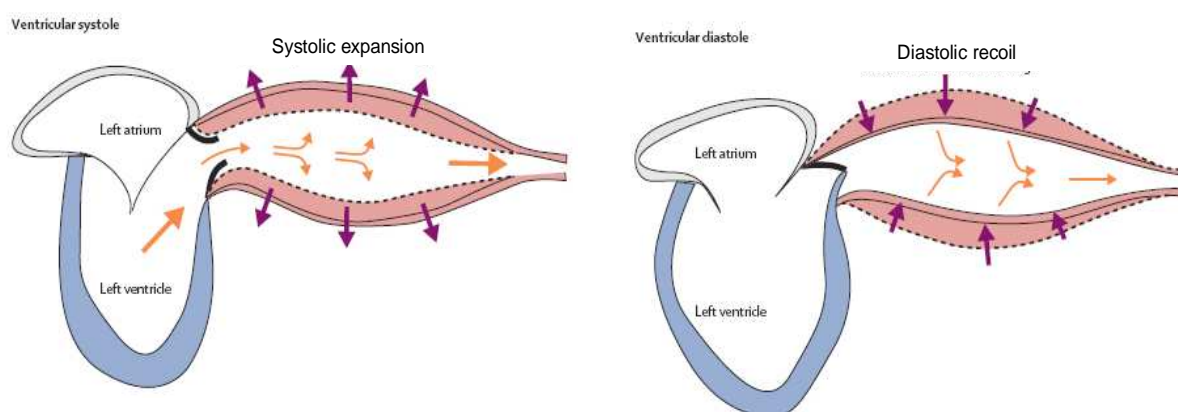
Endothelial dysfunction in hypertensive patients has been associated with target organ damage, such as increased intima-media thickening of the carotid arteries ¹⁸. Moreover, several clinical trials have shown that endothelial dysfunction, evaluated in both coronary and peripheral circulation, is associated with increased incidence of cardiovascular events in high-risk patients ¹⁹. Moreover, FMD is inversely associated with cardiovascular risk in low risk populations ²⁰. Thus, endothelial dysfunction is increasingly being recognized as an

independent predictor of future cardiovascular events and strategies aimed to improve endothelial function may be effective in reducing cardiovascular risk of hypertensive patients²¹.

Arterial Stiffness

In the last decade, there has been a growing awareness that large arterial stiffness is an important marker in the development of cardiovascular disease. During a person's life, as part of the ageing process or due to other cardiovascular risk factors, the aorta stiffens. The capacity of the large vessels to dampen the pulse waves after ventricular ejection diminishes and accordingly the forward pulse wave travels faster, reaching earlier the periphery arterial tree (figure 3).

Figure 3 - Increase of aortic stiffness with aging



This results in increased and precocious reflected waves, which progressively reach the heart in late systole rather than in diastole. This process subsequently leads to higher systolic but lower diastolic blood pressure, therefore increasing the pulse pressure, decreasing diastolic perfusion and augmenting cardiac after load²².

The clinical assessment of arterial stiffness has been defined, described and measured in different ways²³. A current widely used technique to evaluate large artery function is non-invasive pulse wave analysis and pulse wave velocity²⁴. The aortic pulse wave velocity or carotid- femoral pulse wave velocity (CFPWV) is known to be the more direct measure of

arterial stiffness and reflects central stiffness. CFPWV is the measurement of the speed of the pressure waves along the carotid and femoral arterial segments. CFPWV is calculated as the distance divided by the travel time of the wave between 2 sites of the pulse (figure 4). It is furthermore possible to measure peripheral pulse wave velocity (PPWV) by measuring at the radial and carotid artery sites.

Pulse wave analysis (PWA) can be done to obtain data on the augmentation index (AIx) which is a representative surrogate of wave reflection, defined as augmented pressure due to wave reflection divided by the pulse pressure (figure 5). AIx is influenced by the amplitude and timing of forward and backward traveling pressure waves^{25, 26}. Finally, widened pulse pressure is an indirect indicator of increased arterial stiffness.

Figure 4 - Measurement of Central (aortic) Pulse Wave Velocity

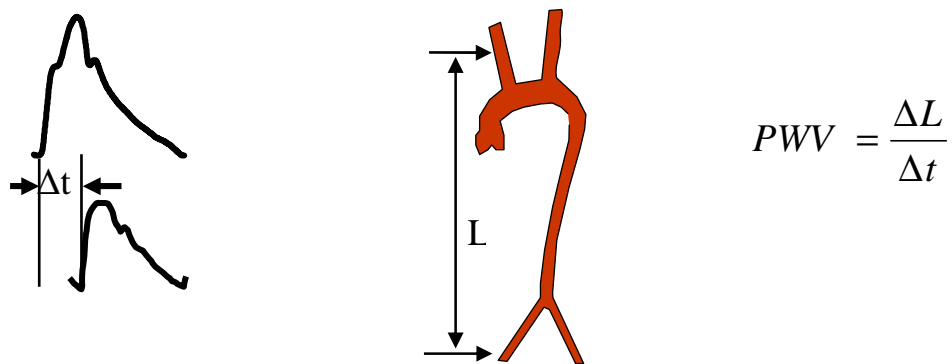
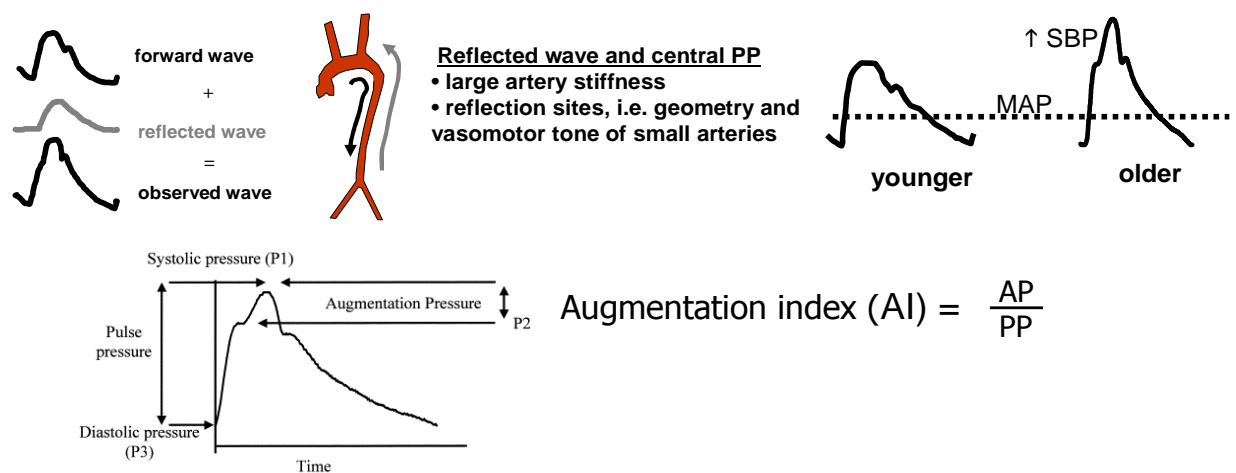


Figure 5 - Measurement of Augmentation index and central pressure



Both the indexes of PWV and PWA are subject to different confounders. Age is one the most powerful predictors of cardiovascular disease and different studies have already demonstrated that age is associated with arterial stiffness^{27, 28}. Some authors suggest that this relation is linear, whereas others now imply that the relation changes with increasing age^{27, 28}. Female subjects in general have higher levels of both PWV and AIx, and this could be in part explained by the fact that women are on average shorter and have smaller and stiffer blood vessels resulting in an earlier return of the reflected wave²⁹. Heart rate has been associated with increased PWV and with lower reflected wave pressure, resulting in a lower AIx²⁷. Therefore in research on arterial stiffness, heart rate as well as gender has to be taken into consideration as a possible confounder especially in research topics such as physical activity.

Hypertension has been associated with increased arterial stiffness and wave reflection^{30 31}. Sustained elevations in blood pressure can also lead to large artery stiffness and this in turn can accelerate the development and progression of systolic hypertension^{31, 32}. This pathological process contributes to hypertensive target organ damage^{33, 34} with a subsequently increase in morbidity and mortality^{30, 33}.

The metabolic syndrome is associated with an increased progression of aortic stiffness with age³⁵. Moreover, some studies have shown already that the metabolic syndrome is associated with an increased arterial stiffness in hypertensive patients³⁶⁻³⁸, although this alteration is more evident for PWV as compared to AIx and in the female gender³⁹.

Longitudinal epidemiological studies have demonstrated the independent predictive value of arterial stiffness and AIx for CV events. The largest amount of evidence has been given for aortic stiffness, measured through CFPWV. Aortic stiffness has independent predictive value for all-cause and CV mortalities, fatal and non-fatal coronary events, and fatal strokes in patients with uncomplicated essential hypertension^{30, 40}, type 2 diabetes⁴¹, ESRD^{42, 43}, elderly subjects^{44, 45} and the general population^{46, 47}. Thus, it is now well accepted that aortic stiffness is an intermediate endpoint for CV events. Central AIx and pulse pressure, measured either directly by carotid tonometry⁴⁸ or estimated using a transfer function from radial artery tonometry^{49, 50} are both independent predictors of all-cause mortality in ESRD patients^{48, 51} and CV events in the hypertensive patients of the CAFE' study⁵⁰. Therefore, strategies aimed at lowering arterial stiffness may be effective in reducing cardiovascular risk.

Non-pharmacological interventions

In the battle for modifying cardiovascular risk factors and eventually in the prevention and reduction of CVD, the most important and studied non-pharmacological interventions are aimed at increasing physical activity and modifying dietary intake. A lifestyle combining regular daily physical activity and a healthy diet is recommended in maintaining body weight and should be the first approach in preventing, control and treatment of CVD.

Physical activity

In recent years there has been a focus on the benefits of physical exercise on cardiovascular disease. Long-term epidemiological studies have shown an unequivocal and robust relation of fitness, physical activity and exercise to reduced mortality overall and from cardiovascular causes and reduced cardiovascular risk ^{52, 53}. Recent investigations have revealed great reductions in the risk of death from any cause and from cardiovascular disease. Being fit or active has been associated with a decrease in risk as great as 50% ⁵⁴. The evidence of the effectiveness of regular physical activity in the primary and secondary prevention of CVD, but also other chronic diseases such as diabetes, hypertension, and obesity, is irrefutable. Therefore sedentary behavior is considered a CV risk factor, considering that the prevalence of physical inactivity is higher than that of all other modifiable risk factors.

Physical activity and hypertension

It is well known that life-style habits and modifications are important in the prevention and treatment of hypertension and it has been shown that regular physical exercise exerts positive effects on blood pressure. In the prevention of hypertension, longitudinal studies have indicated that higher levels of physical activity or fitness are associated with a lower incidence of hypertension ⁵⁵. A meta-analysis of 54 randomized trials showed that aerobic exercise is associated with an overall reduction in mean blood pressure, independent of body weight and race ⁵⁶. In addition, a second meta-analysis showed that dynamic aerobic training reduces blood pressure, with a more pronounced effect in absolute values in hypertensive patients than in normotensive subjects ⁵⁷. Finally, the blood pressure reductions through exercise result in a decrease in CHD, stroke and total mortality ⁵⁶. The findings of a follow-up study of 16 years showed that physical activity in individuals with moderate hypertension can

in part counteract on the adverse effects of high blood pressure on the cardiovascular system⁵⁸.

Since hypertension has been associated with early vascular alterations, such as endothelial dysfunction and arterial stiffness, more recently attention has been focused on vascular function and structure in preventing cardiovascular disease.

Physical activity and endothelial function

Studies have already suggested that physical exercise shows associations with endothelial (dys) function. Different intervention studies reveal a correction of endothelial dysfunction with physical training in patients with chronic heart failure^{59, 60} and coronary artery disease^{61, 62}. Similar studies have shown that aerobic exercise training improved conduit and resistance vessel endothelial vasodilator function in middle-aged patients with type 2⁶³ and type-1 diabetes patients⁶⁴ and patients with essential hypertension⁶⁵.

Studies in healthy subjects show an improvement in endothelial function (FMD) in young healthy subjects after aerobic and anaerobic exercise training⁶⁶. In addition, regular aerobic exercise can prevent and even restore the age-associated loss in endothelium dependent vasodilation in previously sedentary middle aged and older men⁶⁷. Regular physical activity in elderly athletes can at least in part prevent the age-induced endothelial dysfunction⁶⁸. However no data is available on the effect of different levels of regular habitual physical activity on endothelial function.

The mechanisms through which physical exercise can improve endothelial function are not fully clarified. A role for shear stress, which is a physiologic stimulus for NO release, has been hypothesized, stimulating NO production by an increased expression of nitric oxide synthase. Furthermore, physical activity might ameliorate endothelial function by downregulating sympathetic nervous system and decreasing insulin resistance and glucose intolerance^{69, 70}. However the most important mechanism might be the modulation of oxidative stress, reducing the production of ROS and increasing antioxidant defenses⁷¹⁻⁷³. The latter mechanisms are associated with preserved NO availability.

Physical activity and arterial stiffness

Regarding arterial stiffness, available evidence indicates that physically active adults show attenuation in the age-related increase in arterial stiffness in comparison with their sedentary peers^{74, 75}. Furthermore, an observational study conducted in a cohort of men and women free of cardiovascular disease, demonstrated an inverse association between habitual physical activity and arterial stiffness⁷⁶.

Studies have been conducted to evaluate the effect of high intensity exercise on arterial stiffness. A cross-sectional study showed that older men who performed endurance exercise had lower levels of aortic pulse wave velocity and augmentation index than their sedentary peers⁷⁷. A lower aortic pulse wave velocity was also demonstrated in individuals performing habitual endurance exercise as compared to recreationally active individuals⁷⁴

Intervention studies aimed to improve arterial stiffness showed that a moderate intensity and walking type of physical activity increased carotid arterial compliance in previously sedentary, but healthy middle-aged and older men^{78, 79}. The main causes of arterial stiffening with age are a decrease in elastin and the proliferation of collagen fibers in the vascular wall, together with vasoconstrictor tone exerted by its smooth muscle cells⁸⁰. As mentioned before, physical activity seems to attenuate the age-related changes of the arterial wall. Explanations for the beneficial effect of physical activity on arterial stiffness could be a decrease in sympathetic tone and a possibly improvement in endothelial function. However, these mechanisms have not been fully clarified or studied yet.

In summary, persons who are physically active maintain a more favorable cardiovascular risk profile. Regular physical activity not only lowers blood pressure levels but also attenuates the age-related increase in arterial stiffness, which is increasingly being recognized as independent cardiovascular risk factor. Until the present time however no data is present on the relation between physical activity status and augmentation index, a marker of wave reflection in the arterial tree, especially in patients with essential hypertension. Furthermore, it is not known if physical activity exerts different effects on the vascular system in healthy subjects or in patients with hypertension.

The role of antioxidants

Literature indicates that cardiovascular risk factors such as aging and hypertension are accompanied by the presence of endothelial dysfunction and oxidative stress. Oxidative stress, defined as an excessive amount of oxidative substances, relative to endogenous antioxidant capacity, indeed has shown to be increased in conditions such as hypertension, resulting in a reduced NO-availability in these subjects ^{16, 17}. It has been suggested that antioxidants provide vascular defense against oxidative stress by reducing free radicals and protecting NO from inactivation, therefore exerting its' beneficial effects on vascular function and structure.

In essential hypertension the breakdown of NO, through oxidative stress, results in an ongoing pathogenesis of endothelial dysfunction and atherosclerosis. Lately, it has been suggested that nitric oxide may play a role in regulating arterial stiffness. Studies have shown that substances associated with increase in NO production, including glyceryl trinitrate (GTN), reduce arterial stiffness and wave augmentation, whereas inhibitors of NO, such as L-NMMA, increase indexes of arterial stiffness ⁸¹. As mentioned before, physical exercise e.g. shear stress can counteract on this pathogenesis by stimulating the release of nitric oxide.

Another important mechanism through which exercise can improve vascular function is the antioxidant defense capacity. The antioxidant defense capacity is of vital importance in the protection against oxidative stress. Studies indicate that regular physical activity can preserve and upregulate the antioxidant defense capacity. It was shown that physically active older adults might have an improved intracellular antioxidant defense mechanism ⁷¹, whereas animal studies show that training induces gene expression of muscle anti-oxidant enzymes such as glutathione peroxidase and superoxide dismutase ⁷². Furthermore, regular physical activity was closely related with an increased overall oxidant capacity in healthy subjects ⁷³. On the contrary another study pointed out that the level of habitual physical activity and aerobic capacity have no major influence on the resting balance between radical generation and blood-oxidant potential in healthy older man ⁸². On the whole, it seems that physical activity increases antioxidant capacity, an increase that is becoming more pronounced with aging. However until now it is not fully clear what the relationship is between physical activity, antioxidant capacity and vascular function.

The studies on the effect of physical exercise on cardiovascular risk factors and endothelial function are numerous. It is also indicated that the effect of physical exercise can be significantly magnified by other lifestyle changes such as changes in dietary composition and weight loss. The Diet, Exercise and Weight Loss Intervention Trial (DEW-IT)⁸³ showed that a lifestyle intervention can substantially lower blood pressure and improve blood pressure control. An intervention study using an unrestricted consumption of a low-fat, high-fiber diet and daily exercise showed a dramatic improvement in both, blood pressure, oxidative stress and NO availability in obese men⁸⁴.

Studies have been done on the effect of antioxidant vitamins, in regular foods or as food supplements and the risk of cardiovascular disease. In some observational studies a higher intake of antioxidant vitamins or higher plasma levels of antioxidants was associated with lower risk of myocardial infarction and/or stroke⁸⁵. These effects were demonstrated for carotene, ascorbic acid and tocopherol. However, another study, which measured dietary intake and plasma levels of antioxidant vitamins, provided limited support for the hypothesis that increased intake of antioxidant vitamins decreases the risk of atherosclerosis, measured as carotid artery intima media thickness in a randomly selected community population⁸⁶. In this study no benefit was demonstrated for supplemental antioxidant vitamin use.

It can be hypothesized that high dietary intake of vitamin supplementation or experimental oral supplementation with antioxidants can have beneficial effects on cardiovascular disease especially in patients with essential hypertension. However, there is no data available on the association of regular dietary intake of antioxidant vitamins and endothelial function and arterial stiffness. Different studies have already been conducted on the possible effects of Vitamin E or Vitamin C supplementation on cardiovascular disease. Until the present time though, no data exists on the effect of a combined treatment with Vitamin C and E on arterial stiffness and endothelial function in patients with hypertension.

Aims of the study

The aim of the studies is to investigate the role of the metabolic syndrome in hypertensive patients and to evaluate the effects of physical activity, dietary intake and antioxidants on vascular function and structure, especially in hypertensive patients. In order to address this aim the following research questions were developed:

- To determine the effect of the presence of the metabolic syndrome in hypertensive patients on arterial stiffness and endothelial function.
- To determine the relation between habitual physical activity and augmentation index in hypertensive patients.
- To evaluate the relation between physical activity, antioxidant capacity and endothelial function in healthy subjects
- To evaluate the associations between dietary intake and vascular function and vascular structure in normotensive subject and hypertensive patients.
- To test the effect of supplementation with Vitamin C and E on arterial stiffness and endothelial function in hypertensive patients.
- To determine the relationship between measurement of endothelial function (FMD) and measurements of arterial stiffness (PWA and PWV).

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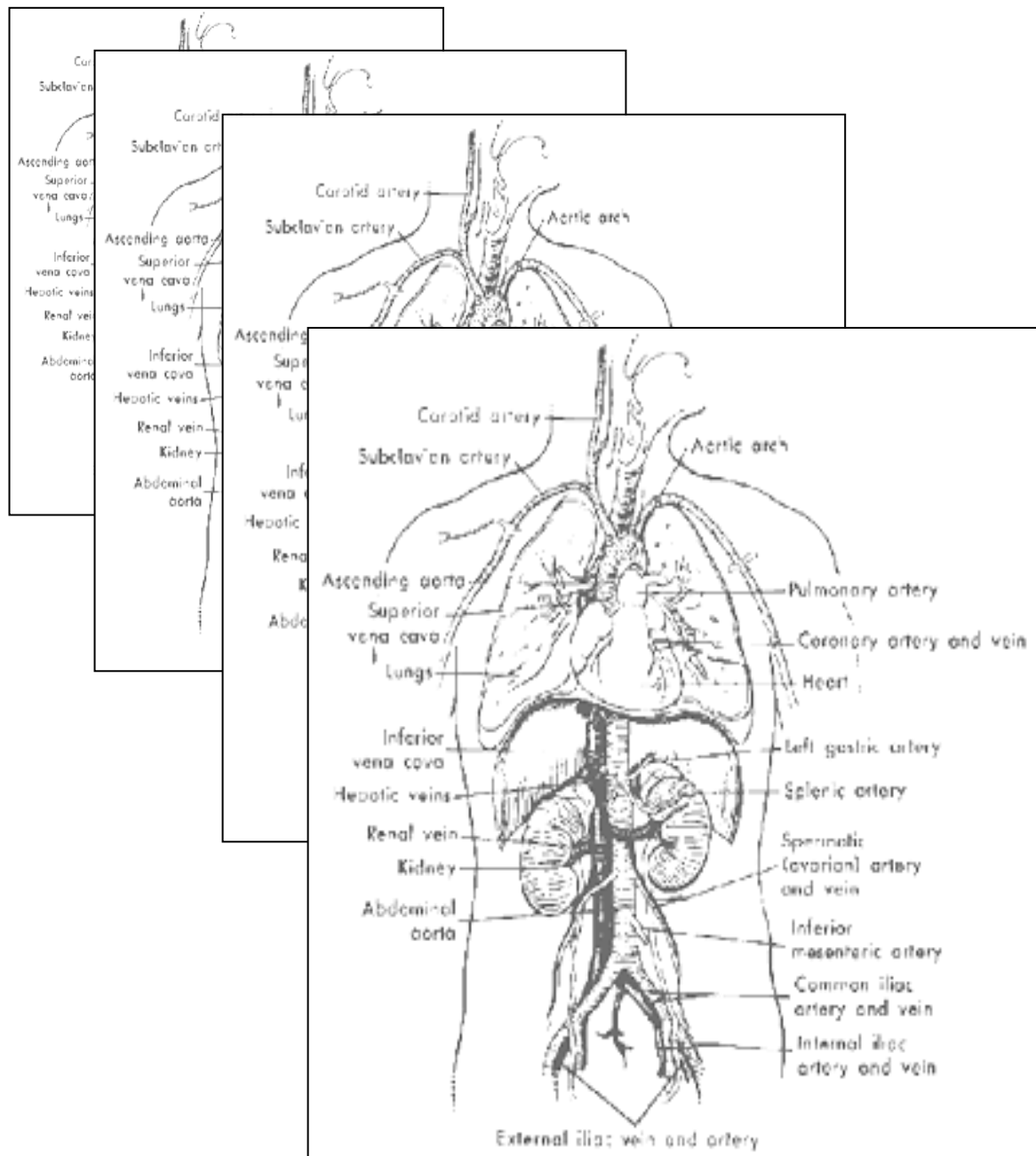
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Chapter 2

**NO DIFFERENCES IN AUGMENTATION INDEX OR
ENDOTHELIAL FUNCTION IN ESSENTIAL
HYPERTENSIVE PATIENTS WITH OR WITHOUT
THE METABOLIC SYNDROME**

Abstract

Background. Essential hypertension is characterized by endothelial dysfunction and arterial stiffness. The metabolic syndrome has been associated with a high risk of cardiovascular disease in hypertensive patients. We evaluated augmentation index, an index of wave reflection and endothelial function in untreated essential hypertensive patients with or without the metabolic syndrome in comparison with normotensive controls.

Methods. Augmentation index was determined with radial applanation tonometry in 232 untreated hypertensive patients and 241 normotensive subjects. Endothelium-dependent response was assessed as flow mediated dilation (FMD) of the brachial artery. The metabolic syndrome (MS) was defined according to the latest National Cholesterol Education Program Adult Treatment panel III (ATP III).

Results. No significant differences were found for AIx or FMD between hypertensive patients with or without the MS. In the normotensive control group, no difference was found for AIx or FMD between subjects with or without the metabolic syndrome. From zero to 3 or more risk factors, in both hypertensive and normotensive subjects, no significant trend was seen for AIx or FMD. Multiple regression analysis showed that age, diastolic blood pressure (DBP) and female gender were the significant predictors of AIx and age, DBP and brachial artery diameter for FMD.

Conclusions. In our population of untreated hypertensive patients and normotensive controls, the presence of the metabolic syndrome does not account for a further deterioration of vascular structural and functional parameters. These results support the hypothesis that blood pressure is the most important component of the metabolic syndrome in defining conduit artery endothelial function and AIx in these subjects.

Introduction

The metabolic syndrome is a metabolic disorder consisting of a clustering of cardiovascular risk factors ^{1, 2}. It is known that the metabolic syndrome carries an increased risk for cardiovascular disease¹⁻³ Furthermore, the metabolic syndrome has been associated with a high risk of cardiovascular disease in hypertensive patients ⁴.

It is known that essential hypertensive patients are characterized by premature alterations in vascular structure and function which can lead to cardiovascular complications. Moreover, these vascular changes observed in hypertension are generally considered to be an accelerated form of the changes seen with aging. Indeed, hypertension is associated with increased arterial stiffness ⁵⁻⁷ and endothelial dysfunction ^{8, 9} and these vascular alterations are increasingly being recognized as powerful predictors of cardiovascular disease morbidity and mortality ^{6, 7, 10, 11}.

It has been hypothesized that hypertensive patients in presence of the metabolic syndrome would also show both earlier and more severe structural and functional alterations of the vascular wall. For this reason research has been focused on the impact of the metabolic syndrome on these vascular parameters. Recent studies suggest that the metabolic syndrome is associated with an increased arterial stiffness in hypertensive patients ¹²⁻¹⁴. Regarding endothelial function, it has been demonstrated that the response to acetylcholine in the forearm microcirculation was blunted in insulin resistant hypertensive patients in the presence of the metabolic syndrome as compared to hypertensive patients without the metabolic syndrome ¹⁵. However, in clinically healthy older men, the metabolic syndrome was not associated with impaired endothelial function, measured as flow mediated dilation of the brachial artery ¹⁶.

Until the present time no data is available on the effect of the metabolic syndrome on augmentation index and conduit artery endothelial function in essential hypertensive patients. Therefore, the aim of this study was to evaluate augmentation index, an index of peripheral wave reflection, and endothelial function measured as flow mediated dilation in a population of untreated essential hypertensive patients with or without the metabolic syndrome in comparison with normotensive controls.

Methods

Study population

In this cross-sectional study, 232 (74 females) hypertensive patients (HT) between 20 and 70 years of age were recruited for the study from February 2004 to December 2005. Patients with essential hypertension were recruited from the newly diagnosed cases in our outpatient clinic and were enrolled if sitting clinic arterial blood pressure (after 10 minutes of rest) was consistently found to be greater than 140/90 mmHg, confirmed in two separate occasions within one month according to European guidelines¹⁷. Patients had never been treated (n=162) or had a history of recent or discontinuous treatment (n=70), with a pharmacological washout for at least 3 weeks before entering the study. Subjects with medical history of cardiovascular events, cardiovascular diseases or other diseases requiring medical treatment, were excluded for the study. To serve as control, 241 normotensive subjects (NT) (105 females) were added. Subjects were defined as normal according to the absence of familial history of essential hypertension and blood pressure values below 140/90 mmHg. To avoid the influence of other overt disease, diabetic patients were excluded for the study.

Body weight, height and waist circumference (WC) were measured in all subjects, body mass index (BMI was) calculated as weight/height². Blood samples were collected following an overnight fast to measure lipid and glucose profile. Information on current smoking status (smoker/non smoker) was also collected. Physical activity levels were assessed and controlled with a modified Baecke questionnaire¹⁸. Written informed consent was obtained from each patient.

The metabolic syndrome (MS) was defined according to the latest National Cholesterol Education Program Adult Treatment panel III (ATP III)³. Subjects were considered to have the metabolic syndrome if they had at least three of the of the five following characteristics: 1) serum triglyceride levels ≥ 1.69 mmol/L (150 mg/dL); 2) serum HDL cholesterol < 1.04 mmol/L (40 mg/dL) in men and < 1.30 mmol/L (50 mg/dL) in women; 3) fasting plasma glucose ≥ 5.55 mmol/L (100 mg/dL); 4) waist circumference > 102 cm in men and > 88 cm in women; 5) blood pressure (BP) $\geq 130/85$ mmHg. Hypertensive patients had to fulfill 2 or more of the first 4 criteria.

The whole study group was furthermore divided in four groups on basis of the different components of the metabolic syndrome 1) no risk factor; 2) 1 risk factor; 3) two risk factors and 4) 3 or more risk factors.

Vascular function

Blood pressure (BP) was measured three times at 3 minutes interval by automatic device at the dominant arm (OMRON-950 CP) and was calculated as the mean value of the last two measurements. Radial tonometry was performed by one trained operator (Y.P.). After an overnight fast, measurements were performed with the subjects in supine position in a quiet, air-conditioned room (22-24°C). A hand held probe was placed on the radial artery from the wrist of the dominant arm and 10-15 subsequent images were recorded. Pulse Wave Analysis (PWA) (SphygmoCor, AtCor Medical, Sydney, Australia) was used to transform the radial pressure waveform into the aortic pressure waveform by using a validated transfer function ^{7, 19}. Three successive measurements were recorded. Augmented pressure (AP) was calculated as the difference between the second systolic peak and the first systolic peak and augmentation index (AIx) was calculated as the ratio between AP and pulse pressure (PP). Since AIx in this study correlated with heart rate, values were normalized at a heart rate of 75 beats/min. An intra-observer reproducibility study in our lab showed a coefficient of variation of 14% for AIx.

Endothelium-dependent response was assessed as dilation of the brachial artery to increased flow (flow mediated dilation, FMD), as previously described ^{20, 21}. Briefly, a B-mode scan of the right brachial artery was obtained in longitudinal section between 5 cm and 10 cm above the elbow using a 7.5 MHz linear array transducer, held by a stereotactic clamp to ensure a constant image. B-mode images were triggered to the ECG signal to obtain only end-diastolic frames. Arterial flow velocity was obtained by pulsed Doppler signal at 70° to the vessel with the range gate (1.5 mm) in the center of the artery. A cuff was placed around the forearm just below the elbow and was inflated for five minutes at 250 mmHg and then deflated to induce reactive hyperemia.

Endothelium-independent dilation was obtained by administration of a low dose (25 µg) of sublingual glyceril trinitrate (GTN). Brachial artery diameter (BAD) measurements were performed after studying the acquired frames by the computerized edge detection system ²². Baseline vessel size was considered as the mean of measures obtained during the first minute.

FMD and response to GTN were calculated as the maximal percent increase in diameter above baseline. Doppler flow velocity was measured at baseline and within 15 seconds after cuff release. Volume blood flow was calculated by multiplying Doppler flow velocity (corrected for the angle) by heart rate and vessel cross-sectional area (πr^2). Reactive hyperemia (RH) was calculated as the maximum percent increase in flow after cuff release as compared to baseline flow.

Statistical analysis.

Data are expressed as means \pm SD. Because of skewed distribution of triglycerides, this variable was logarithmically transformed, and is expressed as median and interquartile range. Characteristics of subjects with or without the metabolic syndrome were compared using Student's *t*-tests or non-parametric test if necessary. All further analysis were adjusted for age, gender, smoking status and physical activity status. Differences in outcome parameters, according to the presence and absence of the MS were tested with analysis of covariance in HT and NT groups separately. Separate analysis was made for gender subgroups, divided for hypertension status and the presence or absence of the MS. The effects of the single dichotomized components of the MS according to the ATPIII criteria ³ on the vascular parameters were tested by analysis of covariance. Simple and multiple regression analyses were used to examine univariate and independent correlates of the vascular parameters. Comparison between groups with different risk factors (0, 1, 2, 3 or more) was made by general linear model. Differences were considered statistically significant when $p < 0.05$. All statistical procedures were performed using the Statview program (Abacus Concepts, Inc., SAS Institute, Cary, NC).

Results

The clinical characteristics of the study population are shown in table 1. Within the group of HT patients, 30% (69/232) was defined as having the metabolic syndrome (HT/MS+). Within the NT group 24% (59/241) met the criteria of the metabolic syndrome (NT/MS+).

In HT and NT groups separately, significant differences were shown for BMI, WC, triglycerides and plasma glucose, which were all significant higher in patients or subjects with the metabolic syndrome, whereas HDL resulted lower in patients with metabolic syndrome. No significant differences were found in age, smoking, systolic (SBP) and diastolic blood

pressure (DBP) between individuals with or without the MS in the HT group or in the NT group. Within the HT group, brachial artery diameter was significantly higher in HT/MS+ as compared to hypertensive patients without the metabolic syndrome (HT/MS-), whereas the percentage of female gender resulted lower in HT/MS+.

Table 1 - Clinical characteristics of study population

	HT/MS+ (n=69)	HT/MS- (n=163)	NT/MS+ (n=59)	NT/MS- (n=182)
Age (years)	48.9 ± 10.4	48.2 ± 8.8	49.5 ± 10.4	47.6 ± 8.8
BMI (kg/m ²)	29.0 ± 3.3†	25.5 ± 8.8	29.9 ± 3.7*	26.5 ± 3.4
Waist circumf. (cm)	102.6 ± 8.6†	90.8 ± 9.8	105.6 ± 8.9*	95.3 ± 9.5
Height (cm)	172.2 ± 9.9	171.7 ± 10.1	169.3 ± 9.8	170.3 ± 9.9
Gender (M/F%)	79/21†	63/37	53/47	56/44
Smoking (%)	21	17	20	17
SBP (mmHg)	156.8 ± 15.1	155.2 ± 14.6	129.5 ± 7.9	128.5 ± 9.6
DBP (mmHg)	95.1 ± 7.5	95.7 ± 7.2	79.7 ± 6.2	79.4 ± 6.3
Total cholesterol (mg/dl)	208.9 ± 41.5	204.9 ± 37.6	227.1 ± 48.3*	202.2 ± 35.0
HDL cholesterol (mg/dl)	41.9 ± 11.5†	54.5 ± 13.3	44.7 ± 7.8*	54.3 ± 13.0
Triglycerides (mg/dl)	186.2 (159-217)†	108.5 (73-131)	182.8 (159-256)*	115.4 (65-137)
Plasma glucose (mg/dl)	103.4 ± 23.0†	90.5 ± 9.6	111.9 ± 17.3*	92.8 ± 10.5
BA diameter (mm)	4.91 ± 0.8†	4.40 ± 0.9	4.05 ± 0.9	4.18 ± 0.8
Level physical activity	7.0 ± 1.5	7.2 ± 1.3	7.0 ± 1.2	7.2 ± 1.5

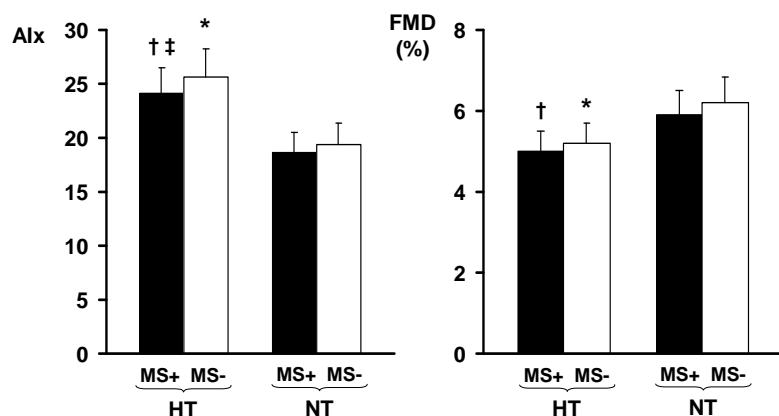
BMI = body mass index; SBP = systolic blood pressure; DBP = diastolic blood pressure;

HDL= high-density lipoprotein; LDL= low-density lipoprotein;

BA diameter= brachial artery diameter. * P<0.05 NT/MS- vs. NT/MS+ and † HT/MS- vs. HT/MS+.

In the whole group, AIx resulted significantly higher in HT as compared to NT (25.5±9.8 vs. 18.4±11.2; p<0.0001), whereas FMD resulted significantly lower (5.0±2.5 vs 6.0±2.7; p<0.001). No significant difference was found in the response to GTN (8.0±3.5 vs 7.9±3.4; p=ns). No significant differences between HT/MS+ or HT/MS- were found for AIx. Furthermore, in the normotensive control group there was no significant difference between NT/MS+ or normotensive subjects without the metabolic syndrome (NT/MS-) (figure 1). A significant higher AIx was observed in HT/MS- compared with NT/MS-, as well as a significant higher AIx in HT/MS+ as compared to NT/MS+. Finally, AIx was significantly higher in HT/MS- as compared to NT/MS+ (figure 1).

Figure 1 - Differences in augmentation index (AIx) and flow mediated dilation (FMD) in hypertensive patients and normotensive subjects with (MS+, black white bars) or without (MS-, white bars) the metabolic syndrome. Data are shown as mean± standard error. * Denotes a significant (p<0.05) difference HT MS- vs. NT MS- (p<0.05); † HT MS+ vs. NT MS+; ‡ HT MS- vs. NT MS+.



In the separate analysis for gender, a significant lower AIx and significant higher values of time to reflection (Tr) and BAD were found in hypertensive female subjects with the MS as compared to female hypertensive subjects in absence of the MS (Table 2). In the normotensive female group, Tr and BAD resulted significantly higher in subjects with the MS as compared to subjects without the MS. In the male subgroup no significant differences were found for any of the vascular parameters (Table 2). All analysis were repeated for central SBP, DBP and pulse pressure (PP), but no significant differences were found for any of these parameters (data not shown).

Table 2 - Age-adjusted values of vascular parameters in male and female hypertensive patients and normotensive subjects according to the absence (MS-) or presence (MS+) of the metabolic syndrome. * $p < 0.05$ MS- versus MS+. Data are shown as mean \pm SE.

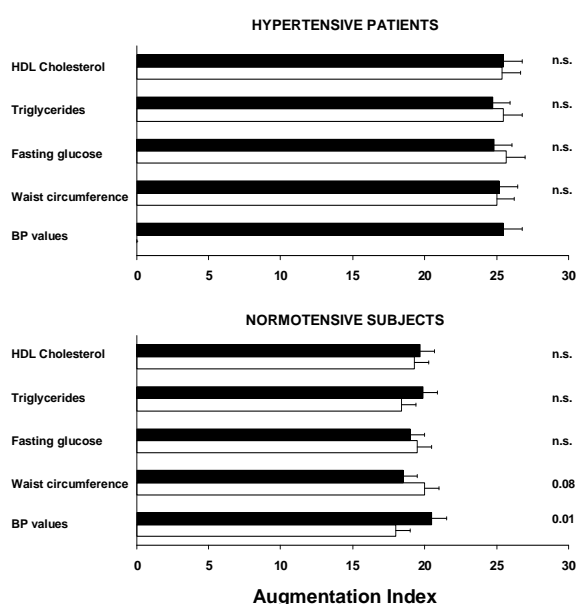
Parameters	Hypertensive patients			
	Males		Females	
	MS+ (n=55)	MS – (n=103)	MS + (n=14)	MS – (n=60)
AIx	22.7 \pm 0.9	22.3 \pm 0.7	26.9 \pm 1.8*	32.7 \pm 0.9
Tr (ms)	143 \pm 3	144 \pm 2	139 \pm 4*	133 \pm 2
cSBP (mmHg)	143 \pm 2	145 \pm 2	148 \pm 4	147 \pm 2
cPP (mmHg)	48 \pm 1	49 \pm 1	54 \pm 3	53 \pm 1
BAD (mm)	4.9 \pm 0.1	4.7 \pm 0.1	4.4 \pm 0.2*	3.9 \pm 0.1
FMD (%)	4.9 \pm 0.3	4.9 \pm 0.3	5.6 \pm 0.8	5.7 \pm 0.4
RH (%)	572 \pm 41	632 \pm 35	653 \pm 69	586 \pm 45
GTN (%)	7.7 \pm 0.5	7.6 \pm 0.3	7.5 \pm 0.9	8.0 \pm 0.4
Parameters	Normotensive subjects			
	Males		Females	
	MS+ (n=36)	MS – (n=100)	MS + (n=23)	MS – (n=82)
AIx	15.4 \pm 1.5	15.1 \pm 0.9	23.1 \pm 1.8	25.3 \pm 1.0
Tr (ms)	154 \pm 4	150 \pm 2	146 \pm 6	140 \pm 3
cSBP (mmHg)	127 \pm 2	124 \pm 1	121 \pm 3	123 \pm 1
cPP (mmHg)	42 \pm 1	41 \pm 1	42 \pm 2	40 \pm 1
BAD (mm)	4.9 \pm 0.1	4.7 \pm 0.1	4.4 \pm 0.2*	3.9 \pm 0.1
FMD (%)	5.7 \pm 0.4	5.8 \pm 0.3	6.4 \pm 0.8	6.7 \pm 0.5
RH (%)	528 \pm 37	569 \pm 32	487 \pm 69	592 \pm 45
GTN (%)	8.6 \pm 0.5	8.5 \pm 0.3	8.4 \pm 0.8	8.8 \pm 0.6

AIx: augmentation index; Tr: time to reflection; cSBP: central systolic blood pressure;
 cPP: central pulse pressure; BAD; brachial artery diameter; FMD: flow mediated dilation;
 RH: reactive hyperemia; GTN: response to sublingual nitroglycerin

Regarding FMD, no significant difference was found between HT/MS+ and HT/MS-, nor between NT/MS+ and NT/MS-. HT/MS- showed a significantly lower FMD as compared to NT/MS- and HT/MS+ showed a significant lower FMD as compared to NT/MS+ (figure 1). Furthermore, no significant differences were found in response to GTN between the groups. Dividing for gender, hypertension status and the presence or absence of the MS, no significant differences in FMD or response to GTN were seen in male and female subjects (Table 2).

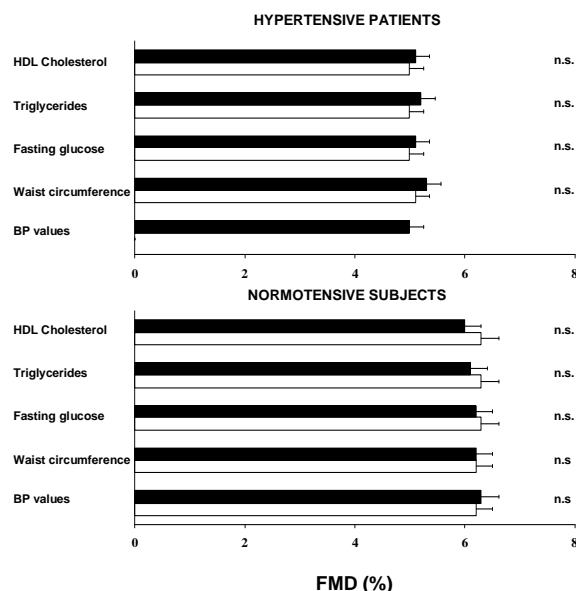
In the analysis evaluating the effect of the dichotomized single components of the MS, no significant differences were seen for AIx or FMD in the hypertensive group (figure 2 and 3). In the normotensive group, FMD was not influenced by any of the dichotomized variables, while AIx was higher in subjects with higher BP values and lower in subjects with higher WC (figure 2 and 3).

Figure 2 - The behavior of augmentation index (AIx) and flow mediated dilation (FMD) according to the presence of 0, 1, 2, 3 or more components of the metabolic syndrome in hypertensive patients (circle) and normotensive subjects (triangle).



In the hypertensive group, simple regression analysis showed that AIx correlated ($p < 0.05$ or less) with age ($r = 0.37$), SBP ($r = 0.20$), DBP ($r = 0.17$), total cholesterol ($r = 0.20$) and inversely with height ($r = -0.43$), BMI ($r = -0.17$) and WC ($r = -0.17$). Finally, FMD correlated negatively ($p < 0.05$ or less) with BAD ($r = -0.43$), age ($r = -0.31$), SBP ($r = -0.16$) and BMI ($r = -0.13$). The multiple regression analysis showed that the predictors of AIx were represented by age, DBP and female gender ($R^2 = 0.56$). For FMD, the significant predictors were age and BAD ($R^2 = 0.23$). When the metabolic syndrome was added as dummy variable in the multiple regression models, the results did not change.

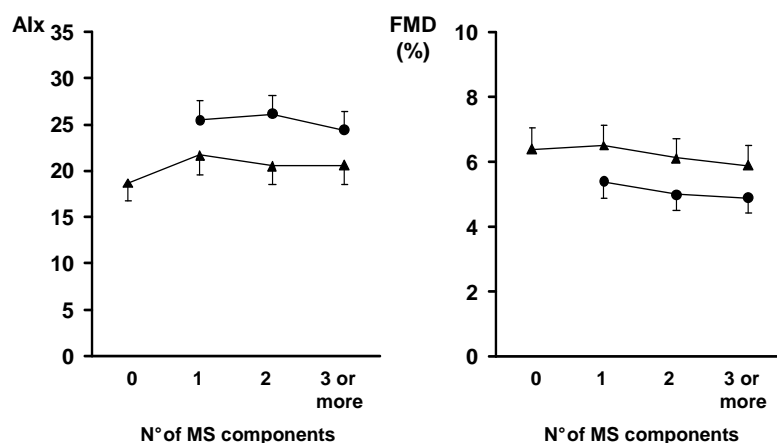
Figure 3 - Differences in augmentation index (AIx) according to the presence (black bar) or absence (white bar) of the single components of the metabolic syndrome in hypertensive patients and normotensive subjects. Mean (standard error)



Univariate regression analysis in the normotensive group showed that age ($r=0.52$), DBP ($r=0.28$) and total cholesterol ($r=0.18$) were positively associated with AIx, whereas height was negatively associated ($r=-0.48$). FMD showed significant inverse associations with age ($r=-0.26$), BAD ($r=-0.43$), BMI ($r=-0.23$), WC ($r=-0.27$) and DBP ($r=-0.16$). Multiple regression analysis showed that age, DBP and female gender were the significant predictors of AIx ($R^2=0.53$). For FMD the significant predictors were BAD, age and DBP ($R^2=0.38$). Adding the metabolic syndrome as a dummy variable in the models did not change the results.

In the overall population, using the general linear model, there was no significant increase in AIx, from zero to 3 or more risk factors in both hypertensive patients and normotensive subjects (fig. 4). For FMD, in the normotensive group, a non significant decrease was seen from 0 to 3 or more risk factors, whereas in the hypertensive patients no decrease was evident (fig 4.). Finally, no trend was seen for GTN in both groups.

Figure 4 - Differences in flow mediated dilation (FMD) according to the presence (black bar) or absence (white bar) of the single components of the metabolic syndrome in hypertensive patients and normotensive subjects. Mean (standard error)



Discussion

The present study demonstrates that in our population, the metabolic syndrome does not account for a further deterioration of vascular structure and function. We showed that AIx, which represents an integrated index of peripheral wave reflection, and FMD, a measure of large conduit artery endothelial function, are not influenced by the presence of the metabolic syndrome both in untreated essential hypertensive patients and in normotensive controls.

Hypertensive patients showed a higher AIx and reduced endothelium dependent dilation as compared with normotensive subjects, irrespective of the presence of the metabolic syndrome. Our data confirm already existing data, showing that hypertension is associated with a reduced endothelium dependent vasodilation ²³, and increased arterial stiffness ⁶ and wave reflection ⁵. Moreover, in the hypertensive patient, AIx was not influenced by any of the single components of the metabolic syndrome, whereas in the normotensive controls only blood pressure levels influenced AIx.

Different studies suggested that the metabolic syndrome is associated with increased arterial stiffness in hypertensive patients. One study showed that central PWV was independently

associated with the metabolic syndrome in untreated hypertensive patients ¹². Increased aortic stiffness, measured as carotid femoral pulse wave velocity, was shown in untreated hypertensive patients with the metabolic syndrome, but this association was largely dependent of age, systolic blood pressure and albumin excretion rate ¹³. Another study showed that the metabolic syndrome was adversely associated with both carotid intima-media thickness and arterial stiffness measured as carotid femoral pulse wave velocity ¹⁴. However in the latter study, blood pressure levels were shown to be the most important component of the metabolic syndrome in defining artery structure and function.

The above mentioned studies indicate that the metabolic syndrome can increase central arterial stiffness, measured as carotid-femoral PWV. The lack of effect of the metabolic syndrome on AIx, indicates a dissociation in the influence of the metabolic syndrome on the various districts or functions of the arterial tree. Interestingly, our results are in line with data obtained in a cohort of treated hypertensive patients, in which AIx was similar in patients with or without the metabolic syndrome ²⁴. Accordingly, one study showed that patients with type-2 diabetes did not have a greater aortic pressure augmentation from peripheral wave reflection when compared with subjects without diabetes ²⁵. Interestingly, in our study population an even lower AIx, together with an increased time to reflection, was found in hypertensive female subjects in presence of the metabolic syndrome as compared to hypertensive females without the metabolic syndrome, confirming a blunted arterial wave reflection in female subjects in presence of the metabolic syndrome ²⁴. In line, it has been hypothesized that adipose tissue at small conduit artery site, can slow wave reflection. This hypothesis was supported by the finding that pressure exerted externally on the hand or leg can reduce wave reflection, resulting in decreased arterial transmural pressure, leaving the peripheral arteries apparently less stiff ²⁶.

Finally, brachial artery diameter was significantly higher in the female subjects with the metabolic syndrome as compared to female subjects without the metabolic syndrome. Since in the multiple regression analysis brachial artery diameter resulted as significant negative predictor of AIx, the before mentioned differences can in part account for our results regarding AIx.

Regarding endothelial function, we did not find any significant difference in endothelial function between subjects with or without the metabolic syndrome. Our results are in line

with a study performed in otherwise clinically healthy older men, which showed that the metabolic syndrome was not associated with impaired endothelial function, measured as flow mediated dilation of the brachial artery ¹⁶. On the other hand it has been demonstrated that the forearm response to acetylcholine in the forearm microcirculation was blunted in insulin resistant hypertensive patients in the presence of the metabolic syndrome as compared to hypertensive patients without the metabolic syndrome ¹⁵.

Importantly, in our study we used the National Cholesterol Education Program (NCEP) Adult Treatment Panel III (ATPIII)³ guidelines to define the presence of the metabolic syndrome, which does not include any measurement of insulin resistance. We can only speculate if the results would be different adding a measurement of insulin resistance.

Moreover, the lack of influence of the metabolic syndrome on the vascular parameters could be explained by the presence of overt disease such as untreated hypertension. In our population of untreated hypertensive patients, the incremental risk attributable, for example, to raised triglycerides or low HDL-cholesterol, is likely to be overwhelmed by the presence of hypertension. Moreover, the study population was screened and recruited on basis of blood pressure levels. Therefore the population resulted heterogeneous regarding the different components of the metabolic syndrome. Furthermore, the hypertensive patients were not extremely overweight and did not show overt dyslipidemia.

The results in the normotensive control group were unexpected. The metabolic syndrome was not associated with higher AIx or lower FMD. However, we showed that within the normotensive control group, higher blood pressure levels were associated with a higher AIx, confirming the major effect of blood pressure levels on this vascular parameter. Accordingly, it is important to underline that AIx was higher in HT patients without the metabolic syndrome as compared to NT patients with the metabolic syndrome. However, since in our control group only 24 % was defined as having the metabolic syndrome, we are cautious in drawing strong conclusions on the effect of the metabolic syndrome in this subgroup.

Increasing age was found to be positively associated with AIx in both hypertensive patients and normotensive subjects, confirming previous observations of a positive association between increasing age and AIx ^{27, 28}. Hypertensive patients however, showed a general higher AIx in both groups as compared to normotensive subjects, suggesting that arterial aging is anticipated in hypertension. Furthermore age was a significant predictor of FMD,

results in line with previous studies, which showed inverse associations between age and endothelial function in the microcirculation⁸ as well as in conduit artery²⁹. The mean age of the study population was 48 years with a range from 20 to 70 years. Since increasing age is an important predictor of both AIx and FMD, we cannot draw conclusions on the influence of the metabolic syndrome in different, especially younger age groups. Indeed, population studies showed that the metabolic syndrome increased the age-related deleterious effects on arterial stiffness in young normotensive adults³⁰ and it was associated with an increased progression of aortic stiffness with age in a 6-years follow-up, supporting premature senescence in these untreated and treated hypertensive patients (80% of the whole population)³¹.

In the whole population another important predictor for AIx was diastolic blood pressure. The clear association of diastolic blood pressure and AIx in both normotensive subjects and hypertensive patients suggests an important role of the microcirculation in determining AIx, therefore confirming that AIx is an index of wave reflection at the peripheral site and subsequently a manifestation of systemic arterial stiffness³².

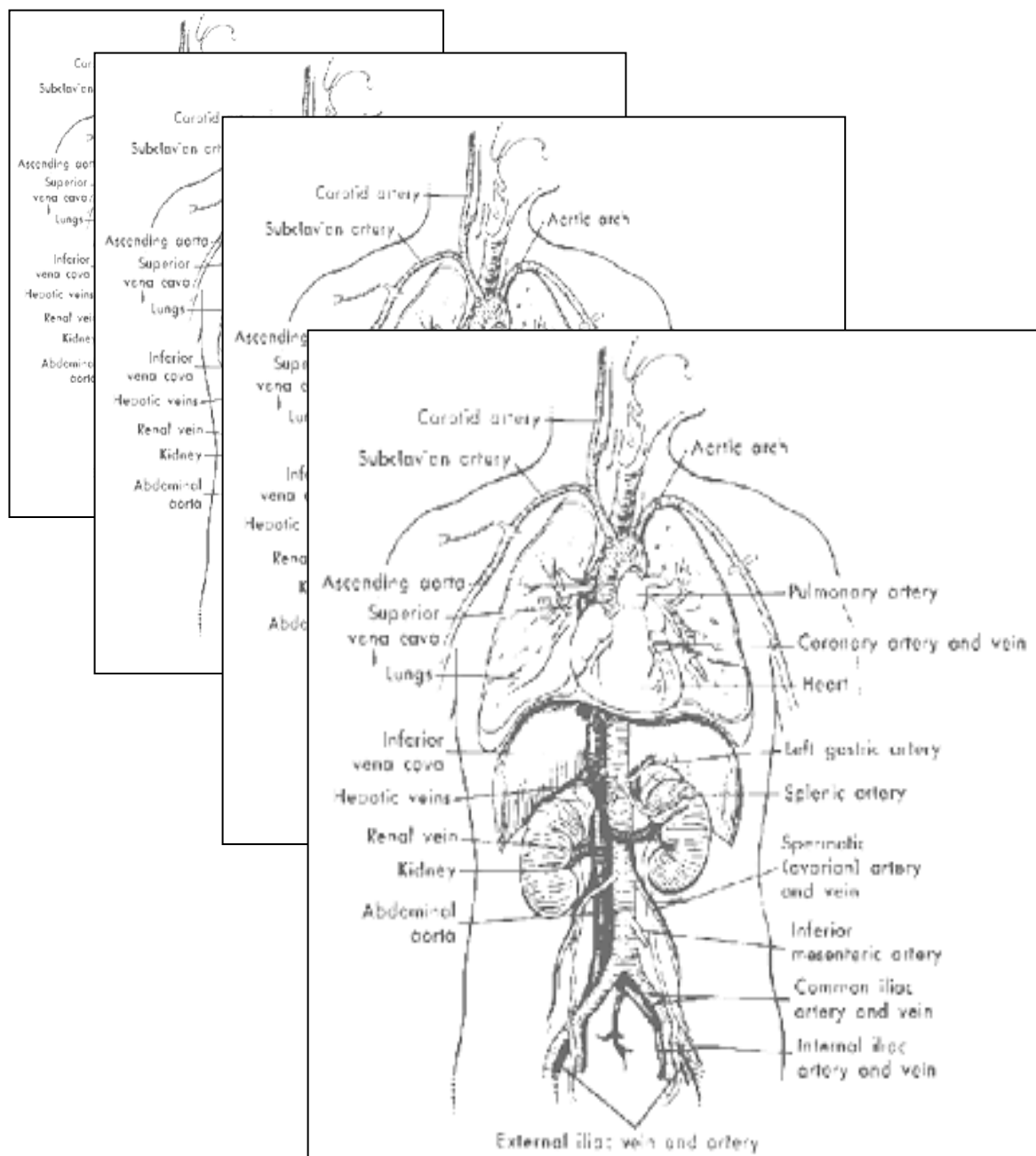
In conclusion, in our population of untreated hypertensive patients and normotensive controls, the presence of the metabolic syndrome does not account for a further deterioration of vascular structural and functional parameters. Beside age and gender, diastolic BP is the only significant predictor of arterial wave reflection. Aging is the main determinant of conduit artery endothelial function. These results support the hypothesis that blood pressure is the most important component of the metabolic syndrome in defining conduit artery endothelial function and AIx in these subjects.

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Chapter 3

HABITUAL PHYSICAL ACTIVITY AND AUGMENTATION INDEX IN UNTREATED HYPERTENSIVE PATIENTS AND NORMOTENSIVE SUBJECTS

Abstract

Background. Hypertension is associated with increased arterial stiffness and wave reflection. Regular physical activity has been shown to lower blood pressure and arterial stiffness in healthy subjects. At the present time no studies have examined the relationship between habitual physical activity and arterial wave reflection in hypertensive patients. The aim of this study was to evaluate the possible associations between habitual physical activity and augmentation index in patients with untreated essential hypertension in comparison with normotensive individuals.

Methods. Augmentation index was determined with radial applanation tonometry in 238 untreated hypertensive patients and 163 normotensive subjects. Habitual physical activity was assessed with a modified Baecke questionnaire, validated by measuring cardio-respiratory fitness in a subgroup (n= 60).

Results. In hypertensive patients, augmentation index did not differ between active and sedentary subjects and none of the physical activity indexes resulted as significant predictor of augmentation index. In the normotensive group, physically active subjects showed significantly lower values of augmentation index as compared to their sedentary peers. Moreover, sport index resulted as an independent predictor of augmentation index.

Conclusions. In normotensive subjects, physical activity was associated with a lower augmentation index, whereas in untreated hypertensive patients this association was not evident. Thus, untreated essential hypertension seems to level out the beneficial impact of habitual physical activity on augmentation index. Further research should investigate the effect of physical activity, in regard with the type and level of physical activity required to exert a beneficial effect in hypertensive patients in the absence or presence of pharmacological treatment.

Introduction

Hypertension is a powerful determinant of cardiovascular risk¹⁻³. Most of the cardiovascular complications associated with hypertension are caused by alterations in vascular structure and function. Indeed, hypertension is associated with increased arterial stiffness and wave reflection^{4,5}. Sustained elevations in blood pressure can also lead to large artery stiffness and this in turn can accelerate the development and progression of systolic hypertension^{5,6}. This pathological process contributes to hypertensive target organ damage^{7,8} with a subsequently increase in morbidity and mortality^{4,7}.

It is well known that life-style habits and modifications are important in the prevention and treatment of hypertension³. Moreover, regular physical activity plays a major role in the prevention and reduction of cardiovascular disease^{9,10}. Longitudinal studies have indicated that higher levels of physical activity or fitness are associated with a lower incidence of hypertension¹¹. It has been demonstrated that aerobic exercise has a blood pressure lowering effect in hypertensive patients as well as in normotensive subjects^{12,13}. Available evidence indicates that in physically active adults the age-related increase in arterial stiffness was attenuated as compared to their sedentary peers^{14,15}. An observational study conducted in a cohort of men and women free of cardiovascular disease, showed a weak association between habitual physical activity and arterial distensibility¹⁶. On the other hand, an intervention trial conducted in a relatively healthy middle-aged population showed that an increased walking frequency was associated with reduced vascular stiffness¹⁷. Finally, in patients with isolated systolic hypertension, short-term aerobic training did not modify large-artery stiffening^{18,19}.

At the present time none of these studies has investigated the effect of habitual physical activity on arterial wave reflection in hypertensive patients. Therefore, the aim of this study was to evaluate the possible associations between habitual physical activity and augmentation index in patients with untreated essential hypertension in comparison with normotensive individuals.

Methods

Study population

In this cross-sectional study, 238 consecutive essential hypertensive (HT) patients between 20 and 70 years of age were recruited for the study from February 2003 to December 2005. Patients with essential hypertension were recruited from the newly diagnosed cases in our outpatient clinic and were enrolled if sitting clinic blood pressure (after 10 minutes of rest) was consistently found to be equal to or greater than 140/90 mmHg, confirmed in two separate occasions within one month, according to ESH-ESC guidelines³. Patients had never been treated (n=159) or had a history of discontinuous treatment (n=79), with a pharmacological washout for at least 3 weeks before entering the study. Patients with prior cardiovascular events, cardiovascular diseases or other diseases requiring medical treatment, were excluded for the study.

One hundred sixty three controls subjects were recruited and defined as normotensive (NT) according to the absence of familial history of essential hypertension and blood pressure values below 140/90 mmHg. The study complied with the declaration of Helsinki of the World Medical Association and the local ethical committee approved the protocol. All study participants gave written informed consent for the study.

Hemodynamics

Blood pressure was calculated as the mean value of three measurements made at 3 minutes interval by an automatic device at the dominant arm (OMRON-950 CP). Radial tonometry was performed by one trained operator (Y.P.). After an overnight fast, measurements were performed with the subjects in supine position in a quiet, air-conditioned room (22-24°C). A hand held probe was placed on the radial artery from the wrist of the dominant arm and 10-15 subsequent images were recorded. Pulse Wave Analysis (PWA) (SphygmoCor, AtCor Medical, Sydney, Australia) was used to transform the radial pressure waveform into the aortic pressure waveform by using a validated transfer function²⁰. Three successive measurements were recorded. Augmented pressure (AP) was calculated as the difference between the second systolic peak and the first systolic peak and augmentation index (AIx) was calculated as the ratio between AP and pulse pressure (PP). Since AIx in this study

correlated with heart rate, values were normalized at a heart rate of 75 beats/min. Coefficient of variation for repeated measures of AIx in our lab is 14%.

Habitual physical activity

Habitual physical activity was measured by interviewing all study subjects with the Baecke questionnaire ²¹. The questionnaire assesses 16 different items concerning habitual physical activity. Responses are coded on a 5-point scale, which provides a guide of general physical activity. The Baecke score is based on three components of physical activity: an occupational/work index, a sport index and a leisure time index. The total index of habitual physical activity was calculated by summing up the individual scores.

To validate the use of the questionnaire in our population, cardio respiratory fitness (VO_2max) was measured in a subgroup of 60 subjects, 29 normotensive subjects and 31 hypertensive patients, with a graded exercise test (cycle ergometer). Initial resistance was set at 25 W and 25 W stepwise increments every 2 minutes. Blood pressure was measured every last 30 seconds of the different steps. No significant differences were found in age- and gender adjusted levels of VO_2 max between HT and NT groups (27.2 and 27.2 ml/min/kg, respectively). VO_2 max was significantly correlated with total index ($R=0.47$; $p<0.01$) and sport index ($R=0.50$; $p<0.01$), but not with leisure time index ($R=0.22$; $p=ns$) and work index ($R=0.10$; $p=ns$).

Blood samples were collected following an overnight fast to measure lipid and glucose profile. Creatinine clearance was evaluated according to simplified MDRD formula ²². Information on current smoking status (smoker/non smoker) was also collected.

Statistical analysis.

Mean levels of selected clinical characteristics in hypertensive patients and normotensive subjects were computed and compared across the two groups by covariance analysis, introducing in each model terms for age and gender. To explore the effect of habitual physical activity, this parameter was added as a categorical variable (active/sedentary) in the analysis, being sedentary classified as the absence of sport activities. Differences between groups (gender and physical activity status) were tested by ANCOVA, adjusting for possible confounders.

Simple regression analysis was used to examine clinical and lifestyle characteristics influencing AIx. Multiple linear regression analysis was performed in the whole study group and separately in HT patients and NT subjects, including in the final model all variables that in the preliminary regression analysis were related to AIx. Differences were considered statistically significant when $p < 0.05$. All statistical procedures were performed using the Statview program (Abacus Concepts, Inc., SAS Institute, Cary, NC) and SAS statistical software (SAS/STAT version 9.1).

Results

Clinical characteristics and physical activity indexes are shown in Table 1 separately for HT patients and NT subjects. Blood pressure values were significantly higher in HT patients than NT subjects, while the differences in gender distribution, age and creatinine clearance were not statistically significant. No differences emerged for BMI, plasma glucose, lipid profile or smoking habits between the two groups. No significant differences were found in levels of total physical activity and sport indexes. The work index resulted higher in HT patients, whereas the leisure time index resulted higher in NT subjects (Table 1).

Table 1 - Age- and gender –adjusted mean values of selected clinical characteristics and physical activity indexes in HT and NT subjects.

	<i>Normotensive subjects</i> <i>N = 163</i>	<i>Hypertensive patients</i> <i>N = 238</i>	P value
Gender (male/female) %	96/64 (39)	161/77 (33)	0.09
Smoking (yes/no) %	31/132 (19)	45/193 (19)	0.98
Age (years)	46.7±10	48.2±11	0.12
Body mass index (kg/m ²)	26.2±2.2	26.2±1.9	0.89
Height (cm)	170.2±9.9	169.6±9.2	0.39
Systolic blood pressure (mmHg)	130±10	155±11	<0.0001
Diastolic blood pressure (mmHg)	81±7	95±7	<0.0001
Total cholesterol (mmol/L)	5.4±0.9	5.3±1.0	0.81
HDL cholesterol (mmol/L)	1.4±0.4	1.4±0.4	0.42
Triglycerides (mmol/L)	1.4±1.5	1.4±1.3	0.78
Plasma glucose (mmol/L)	5.2±0.5	5.2±0.6	0.77
Creatinine clearance (ml/min)	89.0±14.5	86.3±13.9	0.06
Habitual physical activity			
Work index	2.36±0.95	2.53±0.87	0.05
Leisure time index	2.57±0.75	2.44±0.62	0.05
Sport index	2.21±0.80	2.13±0.73	0.30
Total index	7.14±1.50	7.10±1.37	0.80

AIx was significantly higher in HT patients (26.9±9.6%) as compared to NT subjects (20.4±11.3%) (Figure 1). Physically active HT patients did not significantly differ for AIx (26.6±9.4%) as compared to their sedentary peers (27.2±10.6%). According to gender, physically active male and female patients did not significantly differ in age, height, BMI, SBP, DBP, plasma glucose, total and HDL cholesterol. Moreover, physically active male HT patients (n=74) showed similar levels of AIx as compared to their sedentary peers (n=87). Furthermore, no differences were found in AIx between active (n=26) and sedentary (n=51) female HT patients (Figure 2).

Figure 1 - Augmentation index (AIx) in normotensive subjects (left, grey) and hypertensive patients (right, white). Data are shown as median and interquartile range. Bars represent 10th and 90th percentile; dots outliers.

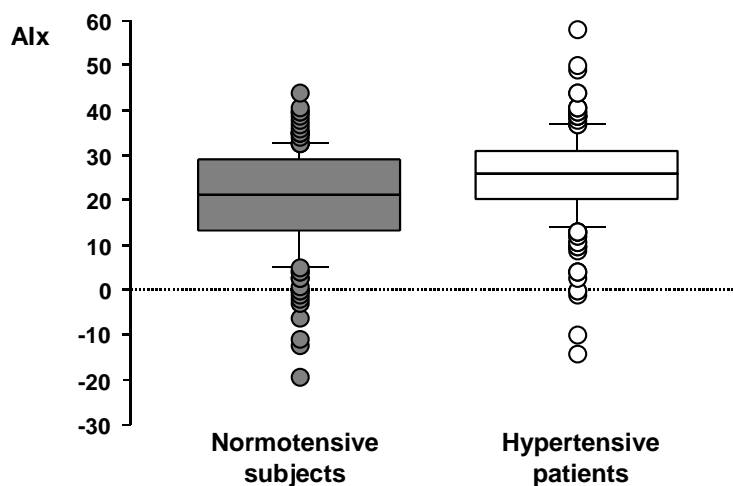
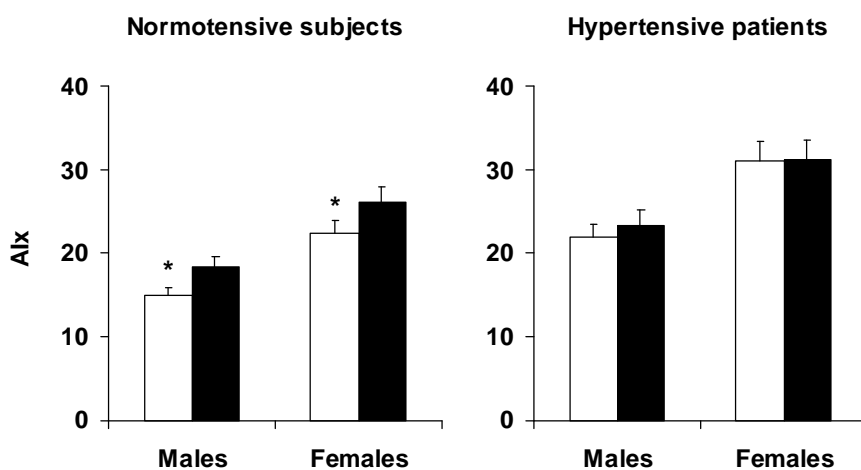


Figure 2 - Adjusted mean values of augmentation index (AIx) among males and females according to physical activity status (active, white bars and sedentary, black bars) in normotensive subjects and hypertensive patients. Data are shown as mean + SEM. * Denotes a significant difference ($p < 0.05$) between active and sedentary groups.



In the NT group, physically active subjects showed a significantly ($p<0.01$) lower AIx ($17.6\pm 9.6\%$) as compared to their sedentary peers ($23.4\pm 10.2\%$). Dividing for gender, physically active male subjects ($n=48$) were significantly ($p<0.05$) younger (43 vs. 48 years) and showed lower BMI (26.1 vs. 28.2 kg/m²), plasma glucose (5.16 vs. 5.34 mmol/L) and higher HDL-cholesterol (1.31 vs. 1.17 mmol/L) as compared to their sedentary peers ($n=48$), while no differences were found for height, SBP, DBP or total cholesterol. Physically active male NT subjects showed significantly lower AIx as compared to their sedentary peers ($p<0.005$). Adjusting for age, BMI, HDL and plasma glucose the difference remained significant ($p<0.05$) (fig. 2). Active female NT subjects ($n=22$) showed lower BMI (23.9 vs. 25.3 kg/m²) and plasma glucose (4.82 vs. 5.11 mmol/L), whereas no differences were found for age, height, SBP, DBP, total and HDL cholesterol as compared with sedentary female NT subjects ($n=45$). Physically active female subjects showed significantly ($p<0.01$) lower AIx as compared to their sedentary peers. Adjusting the values for BMI and plasma glucose the difference remained significant ($p<0.05$) (Figure 2).

Univariate regression analysis showed that age, height, DBP and total cholesterol were significant associated with AIx in NT subjects and HT patients (Table 2). Total physical activity index and sport index of physical activity were inversely related with AIx in NT and HT (Table 2). These associations were more pronounced in NT and the correlation for total index and AIx resulted significantly higher in NT as compared to HT ($p=0.05$). The work index showed an inverse association with AIx only in NT subjects (Table 2). In the validation subgroup, VO₂ max was significantly inversely related to AIx in NT subjects ($R=-0.56$, $p<0.05$), whereas no such relationship was found in HT patients ($R=0.02$, $p=ns$).

Table 2 - Correlation coefficients between selected clinical and lifestyle characteristics and augmentation index.

	Augmentation Index	
	<i>Normotensive n = 163</i>	<i>Hypertensive n = 238</i>
Age	0.52*	0.38*
Height	-0.46*	-0.50*
Systolic blood pressure	0.13	0.20*
Diastolic blood pressure	0.27*	0.17†
Total cholesterol	0.18†	0.20*
Creatinine clearance	-0.14	-0.11
Smoking	-0.12	-0.09
Total index	-0.35*	-0.17†
Work index	-0.20†	0.11
Leisure time index	-0.13	-0.10
Sport index	-0.30*	-0.16†

* p<0.005, † p<0.05.

In the multiple regression models for the evaluation of factors predicting AIx among HT patients, increasing age, DBP, female gender, height and smoking, remained significant predictors, while the sport index was no longer a significant predictor (Table 3). Separate analyses among NT subjects showed that increasing age, DBP, female gender, height and a low sport index (Table 3) were positive predictors of AIx.

In the whole group increasing age (p<0.0001), female gender (p<0.0001), presence of hypertension (p<0.0001), height (p<0.001), low total and sport index (p<0.05) all resulted significant positive predictors of AIx.

Table 3 - Predictors of augmentation index in the two separate categories based on a single multivariate model (upper panel) and a similar model including 3 separate terms for physical activity indexes instead of a total single index (lower panel)

	<i>Normotensive subject</i>		<i>Hypertensive patients</i>	
	β	p	B	p
Gender (male)	-6.31	<0.01	-6.18	<0.0001
Age	0.44	<0.0001	0.34	<0.0001
Height	-0.20	<0.05	-0.23	<0.01
Systolic blood pressure	-0.13	0.18	-0.02	0.62
Diastolic blood pressure	0.57	<0.0001	0.38	<0.0001
Total cholesterol	0.02	0.31	0.02	0.10
Creatinine clearance	-0.02	0.64	0.03	0.39
Smoking	-0.51	0.78	3.55	<0.01
Total index	-0.70	0.14	-0.44	0.22
Work index	0.28	0.70	-0.03	0.95
Leisure time index	-0.64	0.56	-1.04	0.18
Sport index	-1.93	<0.05	-0.33	0.64

Discussion

The aim of this cross-sectional study was to evaluate the possible associations between habitual physical activity and AIX, an index of arterial wave reflection and arterial stiffness in untreated HT patients in comparison with NT individuals.

HT patients showed a higher AIX than NT subjects, a result in agreement with previous studies^{5, 8}. When HT patients were divided according to habitual physical activity status, no difference was found in AIX between active and sedentary subjects. This result remained the same after performing separate analysis for male and female patients. Furthermore, in the HT group the different modalities of habitual physical activity were weakly associated with AIX and none of the physical activity indexes resulted as significant predictor of AIX in the multivariate analysis.

On the contrary in the NT group, physically active males and females showed significantly lower values of AIX as compared to their sedentary peers. Moreover, AIX was significantly related with total index of habitual physical activity, work index and sport index, the latter

resulting as an independent predictor of AIx in the multivariate analysis. Therefore, the major finding of this study is that while among NT subjects physical activity status is associated with attenuation in AIx, in untreated HT patients this association is not present, despite the same level of physical activity in the two groups.

To our knowledge this is the first study to investigate the relationship between habitual physical activity and arterial wave reflection in HT patients. Available intervention studies aimed to improve arterial stiffness were performed in small groups of patients with isolated systolic hypertension^{18, 19}. It was shown that short-term, physical activity of moderate intensity did not change systemic arterial compliance or aortic pulse wave velocity (PWV) in female and male patients¹⁸. Similarly, aerobic exercise did not affect aortic PWV and carotid AIx in post-menopausal women¹⁹. Therefore, the negative results in the HT patients of our study support the evidence that moderate physical activity might not be sufficient to improve arterial stiffness or wave reflection in patients with hypertension. Since sustained untreated or poorly treated elevations of blood pressure increase arterial stiffness²³, it is conceivable that the degree of physical activity is not effective to counterbalance the increase in AIx in HT patients.

On the other hand, results obtained in the group of NT subjects are in line with a study conducted in a cohort of young adults, showing a relationship between sport-related physical activity and aortic PWV²⁴. The latter association was strongly mediated by cardio-respiratory fitness. Furthermore, a cross-sectional study conducted in a cohort of men and women free of cardiovascular disease, showed a weak association between habitual physical activity and carotid distensibility¹⁶. Finally, one study showed that the age-related increase in arterial stiffness was not observed in highly physically active women¹⁵.

Studies conducted to evaluate the effect of high intensity exercise on arterial stiffness showed that older men who performed endurance exercise had lower levels of aortic PWV and AIx than their sedentary peers¹⁴. A lower AIx was also demonstrated in competitive endurance athletes as compared to recreationally active individuals²⁵. Finally, concerning intervention studies, it has been demonstrated that a moderate intensity and walking type of physical activity increased carotid arterial compliance in previously sedentary, but healthy middle-aged and older men²⁶.

Sport index was the most important predictor of AIx among the indexes of habitual physical activity, suggesting that sports' more than work or leisure time activities have an impact on vascular function. The level of physical activity associated to lower AIx in active NT subjects corresponds to moderate-intensity sport activities (jogging, aerobics, cycling and swimming) at a mean level of 3 hours per week, which is in agreement with the current guidelines recommendations²⁷.

Possible explanations for the beneficial effect of physical activity on AIx could reside in a protective effect on conduit artery stiffness and/or vascular reactivity of the microcirculation, leading to a different site of wave reflection. These mechanisms could be mediated by a decrease in sympathetic tone and improvement in endothelial function. In this line, it has been shown that sympathetic activation could alter the compliance of the arterial system²⁸ and it is well established that physical activity is able to lower sympathetic activation^{29, 30}. Moreover, it was shown that regular physical activity in elderly athletes prevented in part the age-induced endothelial dysfunction in conduit arteries³¹ and the forearm microcirculation³². The before mentioned mechanisms could alternatively explain the lack of association of physical activity and AIx in hypertensive patients, since hypertension has been associated with an acceleration of endothelial dysfunction³³ and increased sympathetic nerve activity³⁴.

Important confounders for AIx were age, gender, and diastolic blood pressure. Increasing age was related to higher AIx in both HT patients and NT subjects, confirming previous observations of a positive association between increasing age and AIx³⁵⁻³⁷. Furthermore, female gender was associated with higher levels of AIx in both NT and HT groups. Indeed, it is well known that women have smaller and stiffer blood vessels resulting in an earlier return of the reflected wave³⁸. Finally, the association of diastolic blood pressure and AIx in both NT and HT suggests an important role of the microcirculation in determining AIx, therefore confirming that AIx is an index of wave reflection at the peripheral site and subsequently a manifestation of systemic arterial stiffness³⁹.

The major limitation of the present study is that physical activity levels were assessed indirectly by questionnaire. However, the results were confirmed in the relatively small subgroup in which cardio-respiratory fitness was assessed. Moreover, the cross-sectional design of this study does not allow us to draw conclusions on the dose-effect relation of physical activity and AIx in both NT subjects and HT patients.

In conclusion, the present study suggests a protective effect of habitual physical activity on increased wave reflection in NT subjects, whereas the presence of a cardiovascular risk factor such as untreated essential hypertension seems to level out this beneficial effect. Our findings could have implications for the treatment of hypertension. However, further research should be carried out to investigate the effect of physical activity in hypertensive patients, especially in regard with the type and level of physical activity required to exert a beneficial effect on arterial stiffness in the absence or presence of pharmacological treatment.

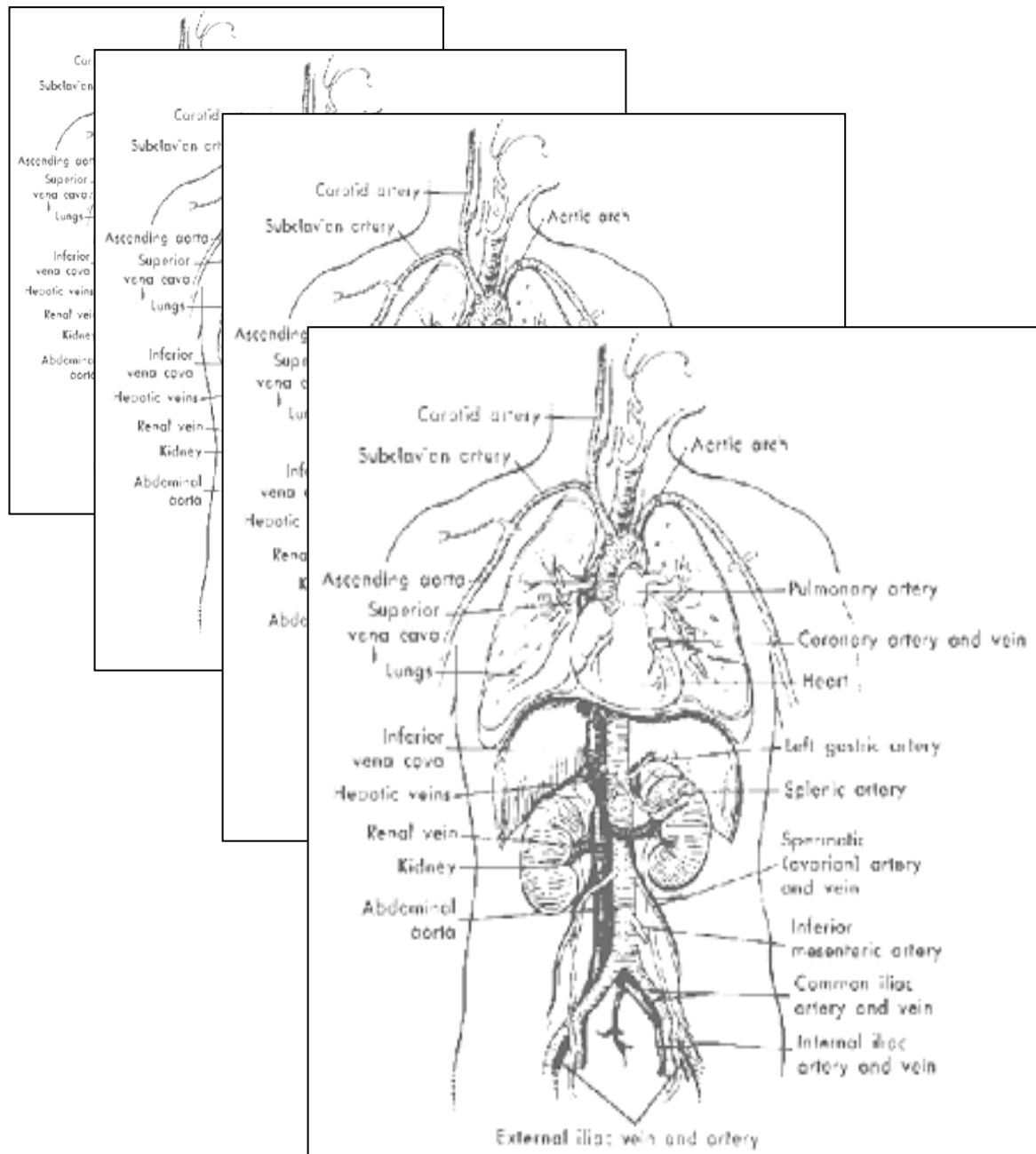
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Chapter 4

PHYSICAL ACTIVITY, PLASMA ANTIOXIDANT CAPACITY AND ENDOTHELIUM-DEPENDENT VASODILATION IN YOUNG AND OLDER MEN

Abstract

Background. Sedentary aging is associated with oxidative stress and endothelial dysfunction. The aim of this study was to evaluate the relationship between chronic physical activity, plasma antioxidant status and conduit artery endothelial function in young and older healthy men.

Methods. In young (n=16) and older athletes (n=16) and matched healthy sedentary subjects, endothelium-dependent flow-mediated dilation (FMD) and endothelium-independent response to glyceryl trinitrate (GTN, 400 µg) were measured in the brachial artery from high-resolution ultrasound. Plasma malondialdehyde (MDA) and antioxidant capacity as Total Oxyradical Scavenging Capacity (TOSC) were also evaluated.

Results. FMD resulted lower (<0.01 or less) in sedentary older subjects ($2.3\pm1.0\%$) as compared with older athletes ($5.3\pm3.2\%$) and both sedentary ($5.4\pm2.0\%$) and trained ($6.1\pm3.2\%$) young subjects. Sedentary older subjects showed higher ($p<0.05$ or less) MDA levels and lower ($p<0.0001$) plasma antioxidant capacity as compared to the other subgroups, while in older athletes MDA levels and antioxidant capacity were similar to those observed in the young subgroups. In the whole group, FMD, but not GTN, was negatively related to age ($r=-0.31$, $p<0.05$) and directly related ($p<0.01$ or less) to VO_{2max} ($r=0.49$) and TOSC against peroxyl ($r=0.69$) and hydroxyl radicals ($r=0.53$). In the multivariate analysis, TOSC against peroxyl radicals resulted as the most significant predictor of FMD ($R^2=0.60$; $p=0.003$).

Conclusion. These results suggest that regular physical activity is associated with preserved antioxidant defenses and endothelial function in older individuals.

Introduction

Endothelium plays an important role in the local regulation of vascular tone and structure, mainly by nitric oxide (NO) synthesis and action.¹ Endothelial dysfunction has been associated with several risk factors for atherosclerosis including dyslipidemia, diabetes, essential hypertension and smoking²⁻⁵.

Aging is an independent risk factor for the development of atherosclerosis and is associated with a progressive decline in endothelium-dependent vasodilation in resistance⁶⁻⁹ and conduit vessels^{10,11}. This alteration is related to a worsening of oxidative status related to both increased oxygen free radicals production^{9,11} and a gradual loss of antioxidant capacity¹², leading together to impaired NO availability⁹.

Physical training has beneficial effects on multiple cardiovascular risk factors, such as dyslipidemia, hypertension, diabetes, and cardiovascular events¹³. The effect of exercise on clinical outcome could also be partially related to a direct and independent positive effect of physical training on endothelial dysfunction in the conduit arteries¹⁴ or in the peripheral microcirculation¹⁵⁻¹⁸. Thus, regular physical activity is associated with an increased endothelium-dependent vasodilation¹⁹ and NO availability¹⁵⁻¹⁸. This beneficial effect is likely to be related to oxidative stress since acute vitamin C administration can improve endothelium-dependent vasodilation in the peripheral microcirculation¹⁷ and conduit arteries¹¹ in old sedentary subjects while ineffective in athletes of the same age. On this regards available evidence supports the possibility that the beneficial effect of physical exercise on oxidative stress might be related to increased antioxidant defenses²⁰.

However no single study has simultaneously addressed the relationship between aging, oxidative stress, antioxidant defenses and physical activity on endothelial function in humans. Therefore, the aim of the present study was to evaluate in young and older athletes and healthy sedentary subjects the relationship between flow-mediated endothelium-dependent dilation (FMD) of the brachial artery, a plasma marker of oxidative stress and plasma antioxidant activity.

Methods

Study population

The study population included young (n=16 males, mean age 33.4 ± 6.7 years) and older athletes (n=16 males, mean age 63.6 ± 6.1 years) and matched healthy sedentary subjects (table 1). Subjects were included in the study in the absence of cardiovascular disease or risk factors. Clinical history, physical examination, basal and stress electrocardiogram, blood chemistry and biochemistry were performed. All the subjects were non-smoking and had no medication or supplementation of vitamins.

Athletes were considered for the study when maximal oxygen uptake (VO_2max), assessed by a graded exercise test (cycle ergometer), was greater than 50 ml/min/Kg, whereas sedentary subjects were included when VO_2max was lower than 45 ml/min/Kg²¹. Young and older endurance-trained subjects performed exercise for 11 ± 2 and 37 ± 5 years, respectively. They performed vigorous endurance exercise (>5 times/week) and were active in national and international road running races and were recruited from running clubs throughout the surrounding regions and from the National Veterans Sport Club of Pisa. Sedentary subjects were recruited through various forms of advertisements. The protocol was approved by the Ethics Committee of the University of Pisa and all patients gave written consent to the study.

Experimental procedure

Vascular ultrasound scans were performed in the morning, with subjects supine, in a quiet air conditioned room (22-24°C). A B-mode scan of the right brachial artery was obtained in longitudinal section between 5 cm and 10 cm above the elbow using a 7.0 MHz linear array transducer and a standard AU5 Armonic system (ESAOTE Biomedica, Florence, Italy) as already described⁴. Briefly, the transducer was held at the same point throughout the scan by a stereotactic clamp. End-diastolic frames were acquired every second on a personal computer using a commercial software program (miroVIDEO DC30/plus, Pinnacle Systems GmbH, Braunschweig, Germany). Arterial flow velocity was obtained by pulsed Doppler signal at 70° to the vessel with the range gate (1.5 mm) in the center of the artery. A cuff was placed around the forearm just below the elbow.

Experimental protocol

Endothelium-dependent response was assessed as dilation of the brachial artery induced by increased flow (flow mediated dilation, FMD)^{2,22}. After 1 minute of baseline acquisition, the cuff was inflated for five minutes and then deflated to induce reactive hyperemia (RH). Endothelium-independent dilation was obtained by sublingual administration of glyceril trinitrate (GTN, 400 µg). Brachial artery diameter was measured on acquired frames by a computerized edge detection system²³. FMD and response to GTN were calculated as the maximal percent increase in diameter above baseline after RH and GTN administration, respectively.

Blood flow volume was calculated by multiplying Doppler flow velocity (corrected for the angle), heart rate and vessel cross-sectional area (πr^2) at baseline and within 15 seconds after cuff release to calculate RH (as percent increase in blood flow).

Blood samples were collected following a 12-h overnight fast. The Total Oxyradical Scavenging Capacity (TOSC) assay was used for measuring the total antioxidant capacity of human plasma against peroxy radicals (ROO·) and hydroxyl radicals (HO·, bottom) by gas-chromatographic analysis (HP 6890 Series, Andoven, Mass.), as previously described^{24,25}. Results were expressed in TOSC units per ml of plasma. In our lab variability of TOSC assay was < 5%²⁶.

Oxidative stress was evaluated by measuring lipid peroxidation of polyunsaturated fatty acids in terms of thiobarbituric acid-reactive substances and converted in malondialdehyde (MDA)²⁷. The amount of lipid peroxidation was reported as micromolar MDA equivalents.

Statistical Analysis

Data were expressed as mean \pm standard deviation (SD). ANOVA method was used to assess the mean differences between groups. Differences were considered significant when $p < 0.05$. Interactions between variables were calculated by correlation and multiple regression analyses. All statistical procedures were performed using the StatView program (Abacus Concepts, Inc., SAS Institute, Cary, NC).

Results

Clinical characteristics of the study population are shown in table 1. Sedentary subjects and athletes were matched for arterial blood pressure and body mass index. According to the inclusion criteria, VO₂max was higher in trained subjects as compared to sedentary subgroups. Athletes also showed a lower resting heart rate as compared to non-athletes. The sedentary older subgroup showed significantly lower HDL and higher LDL cholesterol as compared to the other subgroups.

Table 1 - Clinical characteristics of the study population (mean±SD)

	Young Sedentary Males, n=16	Older Sedentary Males, n=16	Young Athletes Males, n=16	Older Athletes Males, n=16
Age (years)	34.1±7.5	63.7±4.3	33.4±6.7	63.6±6.1
BMI (kg/m ²)	23.1±1.2	24.2±1.3	23.4±0.7	23.9±1.0
Heart rate (bpm)	61.5±4.6	65.3±9.2	49.6±4.3*	53.9±5.2*
SBP (mmHg)	119.3±4.5	126.2±5.4	119.4±4.3	126.3±4.2
DBP (mmHg)	75.3±7.6	78.7±5.8	75.5±3.1	78.2±5.2
Glucose (mg/gL)	91.8±7.7	93.1±9.0	89.6±8.4	92.0±0.9.1
Total cholesterol (mg/dL)	183.9±10.5	187.7±12.0	182.1±9.0	185.9±11.3
HDL cholesterol (mg/dL)	60.5±8.7	43.4±8.7 [#]	63.3±11.1	58.9±11.3
LDL cholesterol (mg/dL)	99.9±12.2	109.4±10.2 [#]	101.7±12.1	101.4±12.8
VO ₂ max (ml/kg min)	37.9±2.9	28.0±5.9 [#]	66.8±2.9°	54.7±3.7
BA diameter (mm)	5.02±0.53	5.07±0.71	5.43±0.57	5.44±0.74
Reactive hyperemia (%)	534±211	513±222	567±190	565±227

BMI = body mass index; SBP = systolic blood pressure;

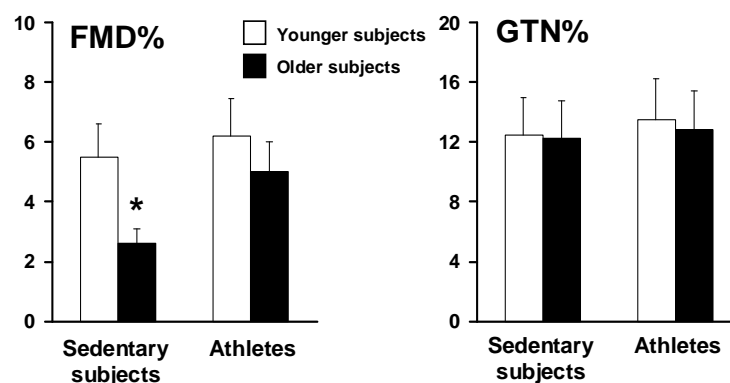
DBP = diastolic blood pressure; VO₂ max = maximal O₂ uptake; BA = brachial artery

*p<0.0001 vs sedentary subgroups, °<0.0001 vs. older subgroups,

[#]<0.0001 vs other subgroups.

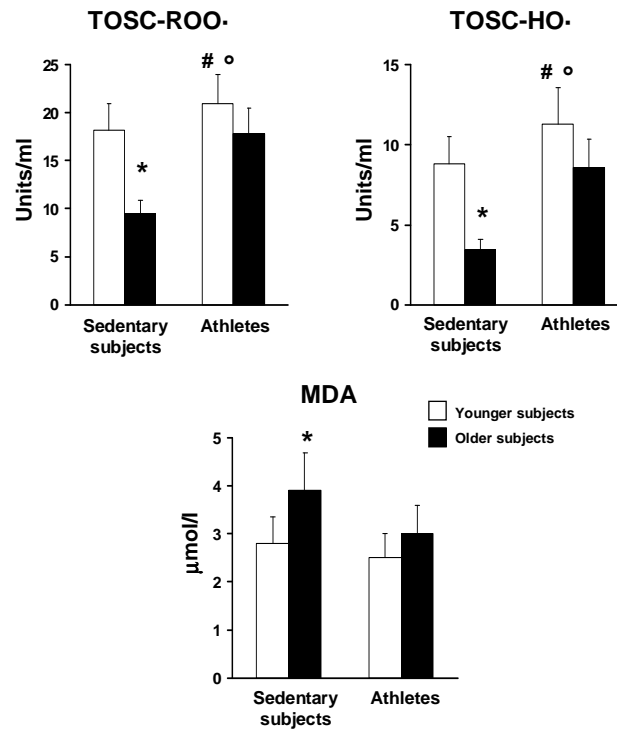
Older sedentary subjects showed lower FMD as compared to older athletes and both young subgroups (figure 1), whereas no differences were shown in the response to GTN (figure 1). FMD, as well as response to GTN, were similar in young and older athletes. RH and brachial artery diameter were similar in the four study groups (table1).

Figure 1 - Flow-mediated dilation (FMD) and glyceryl trinitrate (GTN) induced dilation, expressed as maximal percentage change of brachial artery diameter, in the young and older subgroups of athletes and sedentary subjects (mean \pm SD). * p <0.001 vs other subgroups



Older sedentary subjects exhibit lower TOSC values vs. ROO \cdot and vs. HO \cdot (-48% and -61%, respectively) and higher MDA plasma levels (+26%), as compared to the young sedentary subgroup and both trained subgroups (figure 2). Older athletes showed significantly lower TOSC vs. ROO \cdot and vs. HO \cdot but not significantly higher MDA levels as compared to young athletes, whereas this difference was not shown between older athletes and young sedentary subjects (figure 2).

Figure 2 - Plasma Total Oxyradical Scavenging Capacity (TOSC) against peroxy radicals (ROO•) and hydroxyl radicals (HO•), and plasma malondialdehyde (MDA) in the young and older subgroups of athletes and sedentary subjects (mean±SD). *p<0.001 vs others subgroup, #p<0.01 vs young sedentary subjects, °p<0.01 vs older athletes.



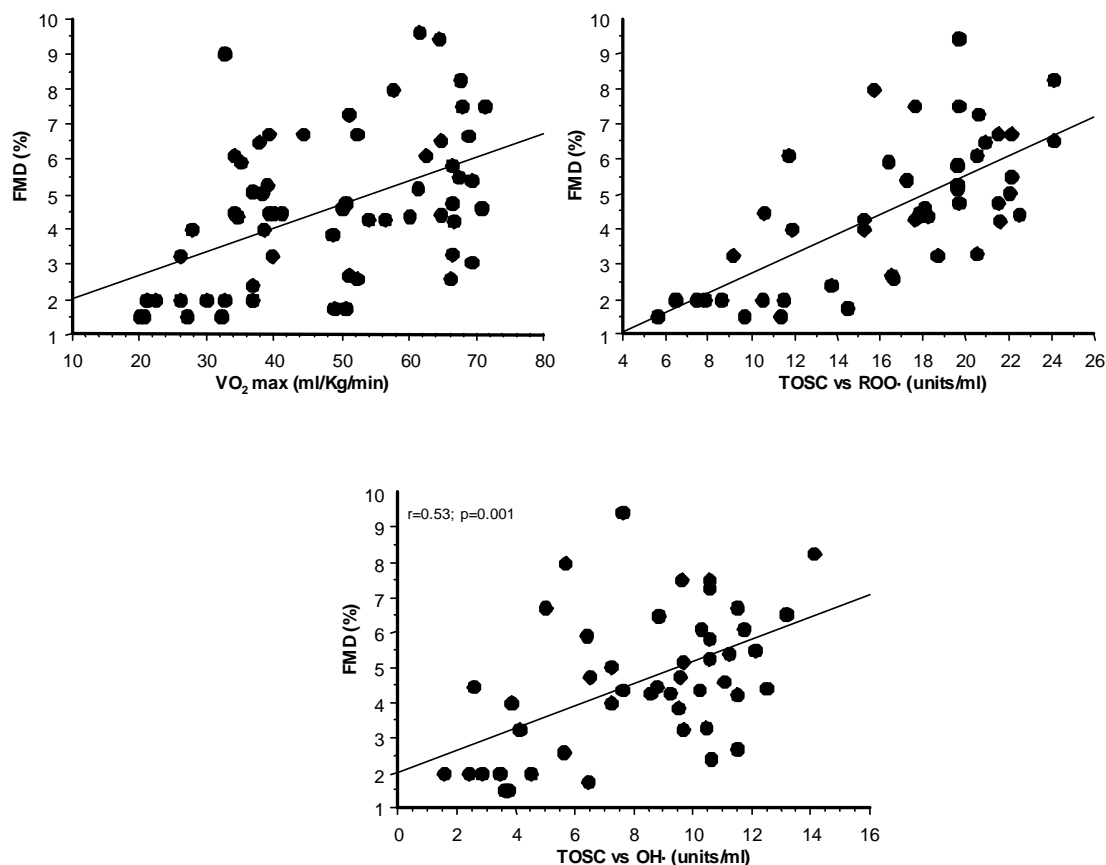
Univariate and multivariate analysis: whole population

In the whole population FMD, but not response to GTN, was significantly related to $VO_2\text{max}$, TOSC vs. ROO• and vs. HO• and inversely related to age ($r=-0.31$; $p<0.05$) (figure 3). FMD was also positively related to HDL cholesterol ($r=0.37$; $p<0.01$) and inversely related to LDL cholesterol ($r=-0.27$; $p<0.05$). $VO_2\text{max}$ was significantly related to TOSC vs. ROO• ($r=0.73$; $p<0.001$) and vs. HO• ($r=0.72$; $p<0.001$) and inversely related to MDA levels ($r=-0.30$; $p<0.05$). Furthermore, age was directly related to MDA ($r=0.44$; $p<0.01$), and inversely related to TOSC vs. ROO• ($r=-0.53$; $p<0.001$), and vs. HO• ($r=-0.57$; $p<0.001$) and $VO_2\text{max}$ ($r=-0.32$; $p<0.05$).

In a multivariate analysis both age and $VO_2\text{max}$ resulted as significant predictors of FMD ($R^2=0.25$; $p=0.0001$). However, $VO_2\text{max}$ accounted for a larger amount of variance in FMD

($R^2=0.13$, $p=0.004$) than age ($R^2=0.07$; $p=0.04$). Including all significant variables, only TOSC value vs. $\text{ROO}\cdot$ resulted as a significant predictor of FMD ($R^2=0.60$; $p=0.003$).

Figure 3 - Correlation analysis between flow mediated dilation (FMD), expressed as maximal percentage change of brachial artery diameter, maximal oxygen uptake (VO_2max , top), and plasma Total Oxyradical Scavenging Capacity (TOSC) against peroxyl radicals ($\text{ROO}\cdot$, middle) and hydroxyl radicals ($\text{HO}\cdot$, bottom).

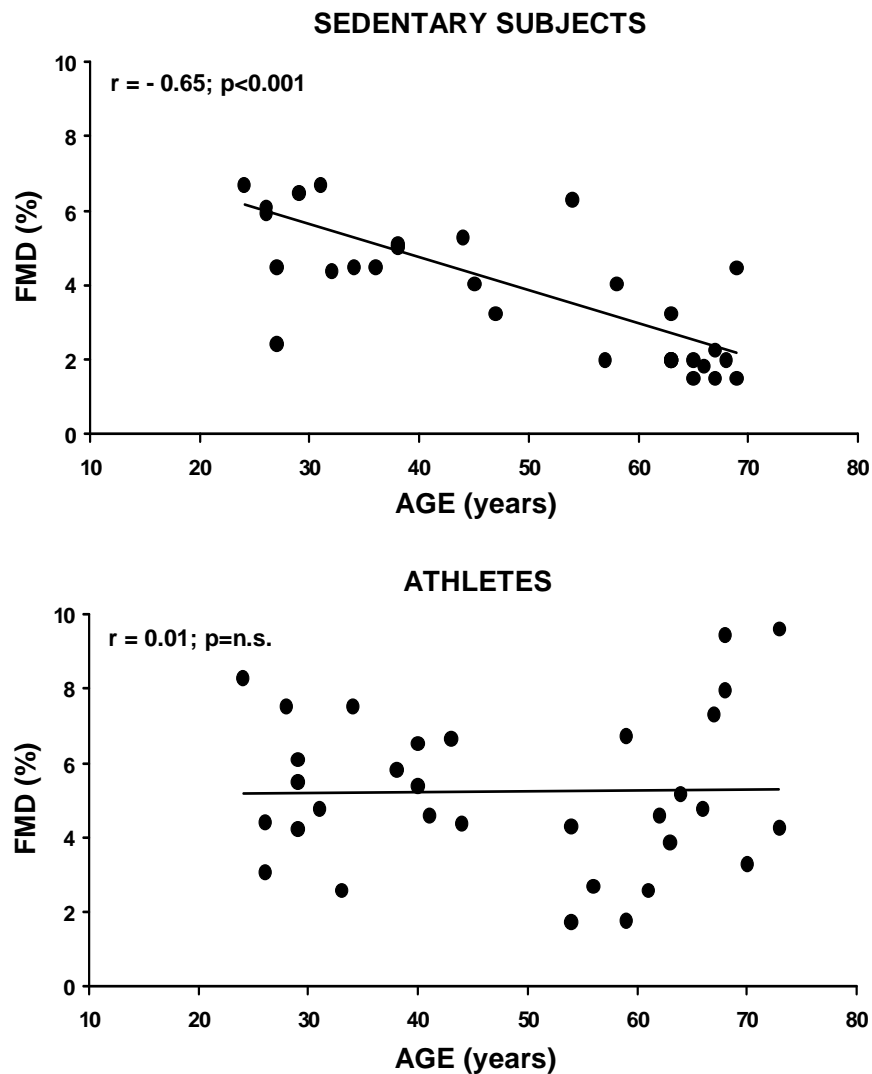


Subgroup's analysis

In the sedentary subjects, FMD was inversely related to age (figure 4). It was also related to VO_2max ($r=0.51$; $p<0.001$), TOSC vs. $\text{ROO}\cdot$ ($r=0.70$; $p<0.001$) and vs. $\text{HO}\cdot$ ($r=0.47$; $p<0.01$) and HDL ($r=0.48$; $p<0.01$). In the multivariate analysis TOSC value vs. $\text{ROO}\cdot$ resulted as the only significant predictor of FMD ($R^2=0.64$; $p=0.02$).

In the athletes, FMD was not related to age (figure 4), while it was related to TOSC vs. $\text{ROO}\cdot$ ($r=0.37$; $p=0.05$) and VO_2max ($r=0.34$; $p=0.05$).

Figure 4 - Correlation analysis between flow mediated dilation (FMD, maximal percent increase) and age in sedentary subjects (top) and athletes (bottom).



Discussion

The present study indicates that advancing age is characterized by an impairment of endothelium-dependent FMD of conduit arteries with a parallel attenuation of plasma antioxidant capacity and increased production of MDA, a marker of oxidative stress. In contrast, in older subjects who regularly perform aerobic endurance training FMD is

preserved. This beneficial effect seems to be related to a higher plasma antioxidant capacity, which can counteract on the oxidative stress production associated with aging.

Older sedentary subjects showed a reduced endothelium-dependent FMD as compared to young subjects, while endothelium-independent vasodilation was similar. These findings confirm previous research showing the presence of impaired endothelium-dependent vasodilation in the brachial artery of older sedentary subjects^{10,11}. This age-related impairment of endothelium-dependent vasodilation could be caused either by a decreased production of NO, due to changes in expression and/or activity of eNOS^{28,29} or an increased degradation of NO, by reactive oxygen species³⁰⁻³².

In the population of sedentary subjects, the older subgroup exhibited a higher MDA, a plasma marker of oxidative stress, as compared to the young subgroup. Moreover, endothelium-dependent FMD was inversely related to MDA, thus supporting the possibility that the age-related increase of oxidative stress could be a promoter of endothelial dysfunction. These results are in line with previous studies showing that ascorbate can acutely restore endothelium dependent vasodilation in the brachial artery¹¹ and in the forearm microcirculation¹⁷ of old sedentary subjects, but not old athletes.

Moreover, aging is associated with a gradual loss of antioxidant capacity¹², which normally provides cellular protection against reactive oxygen species. The evaluation of the TOSC assay, a highly reproducible method for quantitative measurement of the capacity of a molecule or a tissue to neutralize various classes of reactive oxygen species²⁵, showed that plasma antioxidant defense capacity against both peroxyl and hydroxyl radicals is reduced in sedentary aging. In addition, plasma antioxidant defense capacity against peroxyl radicals was only a negative predictor of FMD, suggesting that the loss of antioxidant capacity associated with aging might represent the main determinant of age-related impairment in endothelium-dependent vasodilation.

In line with previous studies^{11,19} older athletes showed a higher FMD than sedentary matched subjects. Moreover, in older athletes FMD was not significantly different in respect to both young subgroups, confirming that endothelial dysfunction is slowed down in older subjects by performing endurance exercise training for many years. Thus, the shown beneficial effects of regular dynamic physical exercise are in line with previous evidence obtained in both conduit arteries¹⁴ and peripheral microcirculation¹⁵⁻¹⁸. However, the interesting and novel finding of

this study is that preserved FMD in older athletes coincides with a higher antioxidant capacity and lower oxidative stress as compared to older sedentary subjects.

Although it has been suggested that acute bouts of exercise can cause oxidative stress by increasing LDL susceptibility to oxidation or vascular superoxide production³³, chronic exercise exerts beneficial effects on oxidative stress ameliorating lipid profile, a phenomenon related to physical activity³⁴ or increasing antioxidant defenses²⁰. In our population maximal oxygen uptake was positively related to HDL and inversely related to LDL cholesterol. HDL-cholesterol was strongly related to plasma antioxidant capacity, supporting the hypothesis that regular endurance training could reduce the susceptibility to oxidative stress by modifying lipid profile. However, since plasma antioxidant capacity against peroxyl radicals was the only significant predictor of FMD, it is conceivable that other mechanisms might account for this effect, including an up-regulation of superoxide dismutase³⁵ and-or other antioxidant defenses^{36,37}.

It is interesting to observe that young athletes have higher TOSC plasma levels as compared to their sedentary peers, while FMD was not significantly different (6,2% vs 5,4%, respectively) in the two subgroups. This discrepancy might be tentatively explained by the possibility that while antioxidant defenses are susceptible to increase, FMD may reach maximum physiological values which is hard to increase. Another explanation could be related to the evidence that individuals undergoing exercise training have high levels of antioxidant enzymes and nonenzymatic antioxidants, causing a greater resistance to exercise-induced or -imposed oxidative stress²⁰. Presumably, these adaptations result from cumulative effects of repeated exercise bouts on the gene expression of antioxidant enzymes²⁰ despite the observed low oxidative level.

In conclusion, preserved antioxidant capacity and endothelium-dependent vasodilation found in older athletes suggest that chronic physical activity can counteract on the age-related endothelial dysfunction which characterizes sedentary aging, probably by maintaining plasma antioxidant defenses and therefore preventing oxidative stress.

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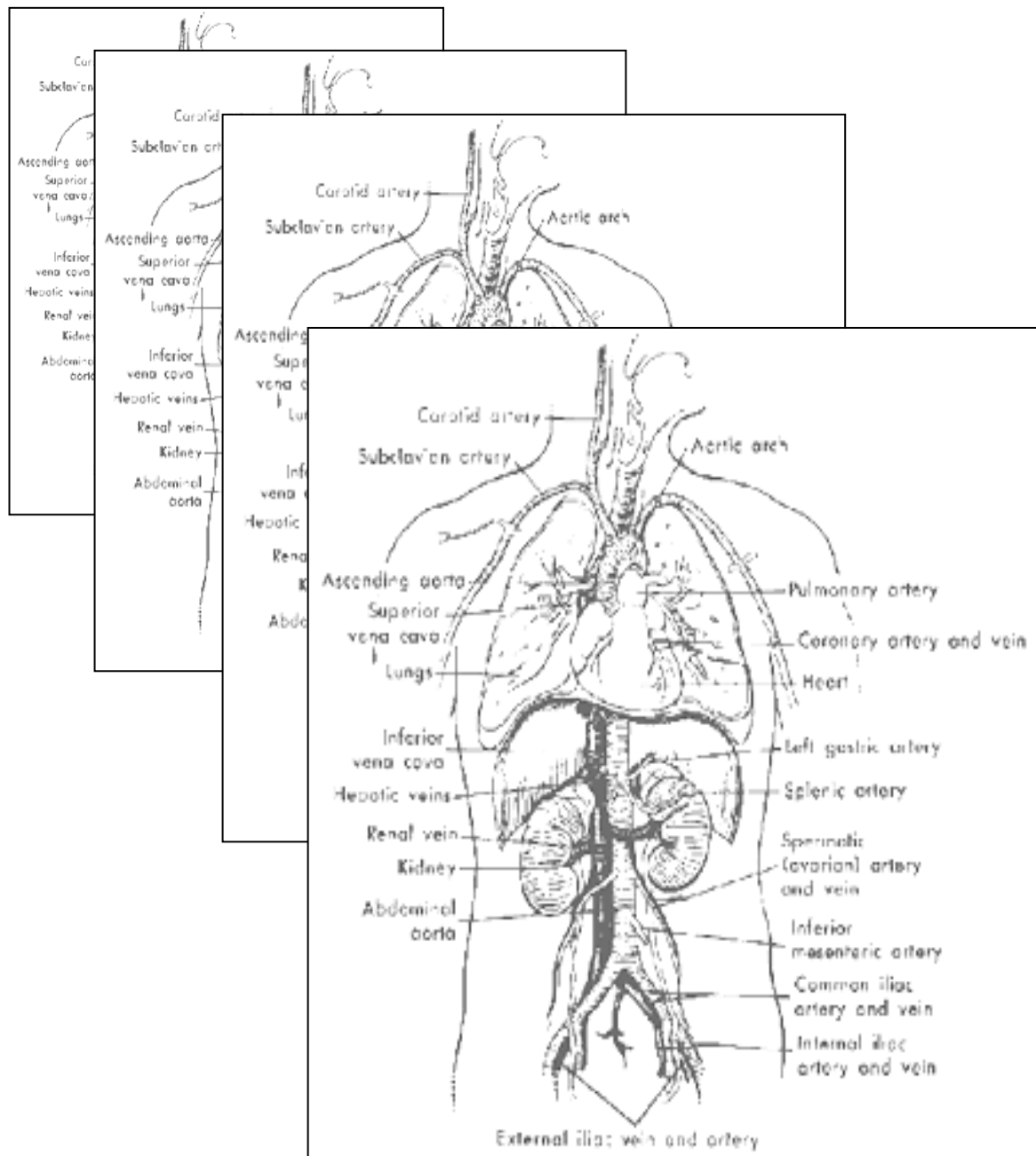
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Chapter 5

REGULAR INTAKE OF ANTIOXIDANT VITAMINS, AUGMENTATION INDEX AND ENDOTHELIAL FUNCTION IN NORMOTENSIVE SUBJECTS AND ESSENTIAL HYPERTENSIVE PATIENTS

Abstract

Background. Essential hypertension is characterized by early vascular alterations such as endothelial dysfunction, increased arterial stiffness and wave reflection. Since these alterations are mediated by increased oxidative stress, the aim of the study was to evaluate the possible associations of daily dietary intake of the antioxidants vitamins C and E with augmentation index and endothelial function in untreated essential hypertensive (HT) patients and normotensive (NT) subjects.

Methods. Dietary intake of major nutrients, total caloric intake and antioxidant vitamins were assessed with the Italian EPIC questionnaires in 196 untreated HT patients and 130 NT subjects. Augmentation index (AIx) was determined with radial applanation tonometry. Endothelium-dependent response was assessed as flow mediated dilation (FMD) of the brachial artery by high ultrasound ultrasounds and computerized analysis (automatic edge detection system) of brachial artery diameter modifications (maximal percentage increase in diameter). Endothelium independent response was assessed by 25 µg of sublingual glyceril trinitrate (GTN).

Results. AIx was significantly higher in HT patients ($26.9 \pm 9.6\%$) as compared to NT subjects ($20.4 \pm 11.3\%$). FMD resulted significantly lower (5.0 ± 2.5 vs 6.2 ± 2.7 ; $p < 0.001$), whereas response to GTN was similar. Estimated intake of vitamin C was quite similar in HT patients and in NT subjects (median 146.4 ± 68.7 mg/day and 149.1 ± 84.0 mg/day, respectively). Intake of vitamin E was also similar (median 8.68 ± 2.7 mg/day and 8.63 ± 2.9 mg/day, respectively). These intakes are in agreement with those commonly consumed in a general Italian population. Multivariate analysis adjusted for smoking, physical activity status and total caloric intake showed that levels of Vitamin C or E intake were not significant predictors for AIx in both HT patients and NT subjects. Moreover, levels of Vitamin C or E intake were not significant predictors for FMD in both groups.

Conclusions. This cross-sectional study showed no association between levels of regular food antioxidant intake, augmentation index and endothelial function in untreated HT hypertensive patients and NT subjects.

Introduction

In the risk assessment of cardiovascular disease, different studies have been done on the possible effects of antioxidant vitamins, in regular food intake or as food supplements. In some observational studies a higher intake of antioxidant vitamins or higher plasma levels of antioxidants was associated with lower risk of myocardial infarction and/or stroke ¹. These effects were demonstrated for carotene, ascorbic acid and tocopherol.

However, other studies, which measured dietary intake or plasma levels of antioxidant vitamins, provided limited support for the association between increased intake of antioxidant vitamins and the risk of mortality² or atherosclerosis, measured as carotid artery intima media ³. A similar study on the effects of vitamin E showed that both intake amount and plasma concentration of vitamin E were inversely associated with preclinical atherosclerosis in middle-aged women ⁴. Finally, in a study, which examined the associations between dietary and plasma vitamin C concentrations, authors found that markers of inflammation and endothelial dysfunction were lower with higher levels of vitamin C intake and plasma vitamin C in older men free of cardiovascular disease ⁵.

Since it has been reported that oxidative stress plays a major role in the atherosclerotic process, it can be hypothesized that dietary intake of vitamins can have different effects on cardiovascular disease or vascular parameters in subjects with or without with increased levels of oxidative stress. In this line, it has been already shown that oxidative stress is increased in conditions such as hypertension ⁶, leading to a lower availability of nitric oxide in these subjects. Moreover, these patients are characterized by endothelial dysfunction ^{7, 8} and increased arterial stiffness ⁹, which both play a role in the atherosclerotic process.

However, until the present time no data is available on the association of regular dietary intake of antioxidant vitamins, endothelial function and a marker of arterial wave reflection, augmentation index in essential hypertensive patients. Therefore, the aim of this study was to evaluate the association of dietary intake of vitamin C and E on these vascular parameters in untreated essential hypertensive patients as compared with normotensive subjects.

Methods

Study population

In this cross-sectional study, 196 consecutive essential hypertensive (HT) patients between 20 and 70 years of age were recruited for the study from February 2003 to December 2005. Patients with essential hypertension were recruited from the newly diagnosed cases in our outpatient clinic and were enrolled if sitting clinic blood pressure (after 10 minutes of rest) was consistently found to be equal to or greater than 140/90 mmHg, confirmed in two separate occasions within one month, according to ESH-ESC guidelines¹⁰. Patients had never been treated (n=139) or had a history of discontinuous treatment (n=60), with a pharmacological washout for at least 3 weeks before entering the study. Patients with prior cardiovascular events, cardiovascular diseases or other diseases requiring medical treatment, were excluded for the study.

One hundred thirty controls subjects were recruited and defined as normotensive (NT) according to the absence of familial history of essential hypertension and blood pressure values below 140/90 mmHg. The study complied with the declaration of Helsinki of the World Medical Association and the local ethical committee approved the protocol. All study participants gave written informed consent for the study.

Hemodynamics

Blood pressure was calculated as the mean value of three measurements made at 3 minutes interval by an automatic device at the dominant arm (OMRON-950 CP). Radial tonometry was performed by one trained operator (Y.P.). After an overnight fast, measurements were performed with the subjects in supine position in a quiet, air-conditioned room (22-24°C). A hand held probe was placed on the radial artery from the wrist of the dominant arm and 10-15 subsequent images were recorded. Pulse Wave Analysis (PWA) (SphygmoCor, AtCor Medical, Sydney, Australia) was used to transform the radial pressure waveform into the aortic pressure waveform by using a validated transfer function¹¹. Three successive measurements were recorded. Augmented pressure (AP) was calculated as the difference between the second systolic peak and the first systolic peak and augmentation index (AIx) was calculated as the ratio between AP and pulse pressure (PP). Since AIx in this study

correlated with heart rate, values were normalized at a heart rate of 75 beats/min. Coefficient of variation for repeated measures of AIx in our lab is 14%.

Endothelium-dependent response was assessed as dilation of the brachial artery to increased flow (flow mediated dilation, FMD), as previously described¹². Briefly, a B-mode scan of the right brachial artery was obtained in longitudinal section between 5 cm and 10 cm above the elbow using a 7.5 MHz linear array transducer, held by a stereotactic clamp to ensure a constant image. B-mode images were triggered to the ECG signal to obtain only end-diastolic frames. Arterial flow velocity was obtained by pulsed Doppler signal at 70° to the vessel with the range gate (1.5 mm) in the center of the artery. A cuff was placed around the forearm just below the elbow and was inflated for five minutes at 250 mmHg and then deflated to induce reactive hyperemia. Endothelium-independent dilation was obtained by administration of a low dose (25 µg) of sublingual glyceril trinitrate (GTN).

Brachial artery diameter (BAD) measurements were performed after studying the acquired frames by the computerized edge detection system¹³. Baseline vessel size was considered as the mean of measures obtained during the first minute. FMD and response to GTN were calculated as the maximal percent increase in diameter above baseline. Doppler flow velocity was measured at baseline and within 15 seconds after cuff release. Volume blood flow was calculated by multiplying Doppler flow velocity (corrected for the angle) by heart rate and vessel cross-sectional area (πr^2). Reactive hyperemia (RH) was the maximum percent increase in flow after cuff release as compared to baseline flow.

Blood samples were collected following an overnight fast to measure lipid and glucose profile. Information on smoking status, educational level and habitual physical activity was also collected.

Dietary intake of antioxidant vitamins

Dietary intake was measured with the EPIC/Italy food frequency questionnaire, which is a validated semi/quantitative, self administered food frequency questionnaire¹⁴. The self-administered questionnaire on dietary intake contains questions on the average consumption frequency during the past year. Subjects could indicate their answers in portion size and times per day, week, month, or as never. With a specific software, total caloric intake, major nutrients and vitamin intake could be determined.

Statistical analysis.

Due to skewed distributions, vitamin C and E levels were log transformed. Mean levels of selected clinical characteristics in hypertensive patients and normotensive subjects were computed and compared across the two groups by covariance analysis, introducing in each model terms for age and gender. Simple regression analysis was used to examine clinical and lifestyle characteristics influencing AIx and FMD. Multiple linear regression analysis was performed in HT patients and NT subjects, and level of vitamin C and E intake separately, including in the model all variables that in the preliminary regression analysis were related to AIx or FMD. Differences were considered statistically significant when $p < 0.05$. All statistical procedures were performed using the SAS statistical software (SAS/STAT version 9.1).

Results

Clinical characteristics and levels of vitamin intake are shown in Table 1 separately for HT patients and NT subjects. Blood pressure values were significantly higher in HT patients as compared to NT subjects, as well as hematocrit levels and brachial artery diameter. Female gender resulted significantly higher in the NT subjects. No differences emerged for age, BMI, plasma glucose, lipid profile or smoking habits between the two groups. No significant differences were found in levels of vitamin C and E intake, total caloric intake and levels of physical activity (table 1). AIx was significantly higher in HT patients ($26.9 \pm 9.6\%$) as compared to NT subjects ($20.4 \pm 11.3\%$) whereas FMD resulted significantly lower (5.0 ± 2.5 vs 6.2 ± 2.7 ; $p < 0.001$).

Univariate regression analysis in the HT group showed that age, gender, height, BMI, SBP, DBP, MAP, total cholesterol, hematocrit levels, total index of physical activity and total caloric intake were significant associated with AIx (Table 2). In the NT group, age, gender, height, BMI, DBP, MAP, hematocrit levels and total index of physical activity were significant related to AIx (Table 2). No significant associations were found for vitamin C and E intake and AIx in both groups. In the multiple regression models for the evaluation of factors predicting AIx among HT patients, increasing age, MAP, female gender, hematocrit levels and current smoking, remained significant predictors (Table 3).

Table 1 - Age- and gender –adjusted mean values of selected clinical characteristics and physical activity indexes in HT and NT subjects.

	<i>Normotensive subjects</i> <i>N = 130</i>	<i>Hypertensive patients</i> <i>N = 196</i>	P value
Age (years)	47.1±10	48.2±11	0.31
Gender (male/female) %	73/57 (44)	133/63 (32)	0.03
Current smoking (yes/no) %	26/104 (20%)	33/163 (17%)	0.21
Ex smoking (%)	32	41	0.21
Body mass index (kg/m ²)	26.0±2.2	26.6±1.9	0.12
Height (cm)	171.3±9.9	171.5±9.2	0.86
Systolic blood pressure (mmHg)	130±10	156±11	<0.0001
Diastolic blood pressure (mmHg)	80±7	96±7	<0.0001
Total cholesterol (mmol/L)	5.4±0.9	5.4±1.0	0.89
HDL cholesterol (mmol/L)	1.4±0.4	1.4±0.4	0.42
Triglycerides (mmol/L)	1.4±1.5	1.4±1.3	0.78
Plasma glucose (mmol/L)	5.2±0.5	5.2±0.6	0.77
Hematocrit (%)	42.6±3.4	43.7±3.7	<0.01
Brachial artery diameter (mm)	4.31±0.77	4.60±0.86	0.002
Total index physical activity	7.3±1.6	7.1±1.3	0.25
Total caloric intake (kcal/day)	2541±801	2613±932	0.47
Vitamin C intake (mg/day)	149.1±84.0	146.4±68.7	0.75
Vitamin E intake (mg/day)	8.63±2.9	8.68±2.7	0.89

Table 2 - Correlation coefficients between selected clinical and lifestyle characteristics and augmentation index.

Variable	Augmentation Index	
	<i>Normotensive n = 130</i>	<i>Hypertensive n = 196</i>
Age	0.48*	0.38*
Gender	-0.45*	-0.52*
Height	-0.46*	-0.51*
BMI	-0.09	-0.19†
Systolic blood pressure	0.17	0.21*
Diastolic blood pressure	0.22*	0.18†
MAP	0.45*	0.40*
Total cholesterol	0.17	0.23*
Hematocrit	-0.22*	-0.39*
Current smoking	0.12	-0.09
Total index physical activity	-0.35*	-0.17†
Total caloric intake	-0.12	-0.14†
Vitamin C (mg/day)	-0.03	-0.01
Vitamin E (mg/day)	-0.08	-0.06

* $p < 0.005$, † $p < 0.05$.

Separate analyses among NT subjects showed that increasing age, MAP and female gender (Table 3) were positive predictors of AIx. In both groups, levels of Vitamin C or E intake were not significant predictors for AIx. In the NT group, β - values for levels of Vitamin C and E intake were both negative for AIx, but this did not reach the level of statistical significance, whereas in the HT group β - values showed a positive trend with AIx (Table 3).

For FMD, univariate regression analysis group showed that age, BMI, and BAD were significantly inversely associated with FMD in both HT and NT subjects (Table 4). No significant associations were found for vitamin C and E intake and FMD in both groups. In the multiple regression models for the evaluation of factors predicting FMD among HT patients, increasing BAD, age, and current smoking remained significant negative predictors (Table 5). Separate analyses among NT subjects showed that increasing age, and BAD (Table 5) were negative predictors of FMD. Finally, in both groups, levels of Vitamin C or E intake were not significant predictors for FMD.

Table 3 - Predictors of augmentation index in the two separate categories based on two separate multivariate models for Vitamin C and E intake.

Variable	<i>Normotensive subject</i>		<i>Hypertensive patients</i>	
	β	p	B	p
Gender (male)	-8.16	<0.0001	-5.78	<0.001
Age	0.28	<0.0001	0.25	<0.0001
Height	-0.14	0.13	-0.18	0.02
MAP	0.51	<0.0001	0.27	<0.0001
Total index physical activity	-0.47	0.29	-0.70	0.12
Total caloric intake	1.73	0.48	0.81	0.65
Total cholesterol	0.02	0.24	0.02	0.11
Hematocrit	0.06	0.79	-0.53	0.002
Current smoking	-0.79	0.64	4.82	<0.01
Ex smoking	2.21	0.14	1.56	0.16
Vitamin C (mg/day)	-1.97 $R^2=0.60$	0.20	2.02 $R^2=0.58$	0.09
Vitamin E (mg/day)	-4.05 $R^2=0.60$	0.18	0.86 $R^2=0.57$	0.73

Table 4 - Correlation coefficients between selected clinical and lifestyle characteristics and flow mediated dilation (FMD) * $p<0.005$, † $p<0.05$.

Variable	FMD	
	<i>Normotensive n = 130</i>	<i>Hypertensive n = 196</i>
Age	-0.31*	-0.33*
Gender	-0.16	-0.08
Height	-0.05	-0.03
BMI	-0.29*	-0.17*
Systolic blood pressure	0.02	-0.13
Diastolic blood pressure	0.12	0.01
MAP	0.01	-0.02
Total cholesterol	-0.08	-0.03
Brachial artery diameter	-0.46*	-0.39*
Current smoking	-0.04	-0.08
Total index physical activity	0.12	-0.02
Total caloric intake	-0.10	0.05
Vitamin C (mg/day)	0.14	0.04
Vitamin E (mg/day)	0.07	-0.06

Table 5 - Predictors of flow mediated dilation (FMD) in the two separate categories based on two separate multivariate models for Vitamin C and E intake.

Variable	<i>Normotensive subject</i>		<i>Hypertensive patients</i>	
	β	p	B	p
Gender (male)	0.43	0.44	0.47	0.27
Age	-0.06	<0.01	-0.07	0.0001
BMI	-0.11	0.08	-0.03	0.52
Brachial artery diameter	-1.70	<0.0001	-1.06	<0.0001
MAP	0.51	<0.0001	0.01	0.52
Total index physical activity	0.20	0.19	-0.24	0.08
Total caloric intake	0.31	0.69	0.81	0.65
Total cholesterol	0.00	0.84	0.00	0.81
Current smoking	-0.77	0.16	-0.85	0.07
Ex smoking	0.67	0.16	-0.27	0.46
Vitamin C (mg/day)	0.57 $R^2=0.38$	0.25	-0.01 $R^2=0.26$	0.99
Vitamin E (mg/day)	-0.50 $R^2=0.38$	0.61	-1.34 $R^2=0.27$	0.12

Discussion

The aim of this cross-sectional study was to evaluate the possible associations between dietary intake of the antioxidant vitamins C and E, AIX, and FMD in untreated HT patients in comparison with NT individuals. The present study demonstrates that levels of vitamin C and E intake were not associated with augmentation index or endothelial function in untreated essential HT patients and NT controls. To our knowledge no previous study has investigated the relationship between regular food intake of the antioxidant vitamins C and E on both augmentation index and endothelial function in essential HT patients. As to be expected, HT patients showed a higher AIX and a lower FMD than NT subjects, a result in agreement with previous studies¹⁵⁻¹⁷.

Our results differ from some observational studies which showed that a higher intake of antioxidant vitamins or higher plasma levels of antioxidants was associated with lower risk of myocardial infarction and/or stroke¹, effects that were demonstrated for carotene, ascorbic

acid and tocopherol. However, in a randomly selected community population, the association between increased intake of antioxidant vitamins and the risk of atherosclerosis, measured as carotid artery intima media thickness was found to be weak ³. A similar study showed that low vitamin E intake was a risk factor for early atherosclerosis, measured as carotid plaques, in middle-aged women ⁴. Finally, in a study, which examined the associations between dietary and plasma vitamin C concentrations, authors found that markers of inflammation and endothelial dysfunction, measures as tissue plasminogen activator (t-PA) antigen were lower with higher levels of vitamin C intake and plasma vitamin C in older men free of cardiovascular disease ⁵.

The fact that in the present study we measured the intermediary endpoints AIx and FMD, and not overt atherosclerosis such as carotid plaques or stronger end-points such as stroke or myocardial infarction can explain the discrepancy between the results. Indeed this is the first study to show that there is no association found between regular antioxidant vitamin C and E intake and the vascular parameters augmentation index, which is a marker of arterial wave reflection, and endothelial function measured as FMD. In the multiple regression analysis we found negative standardized β -coefficients for vitamin C and E intake on augmentation index in the normotensive subjects, which did not reach the level of statistical significance. On the contrary, slightly positive β -coefficients were found in the hypertensive patients, indicating a possible trend for a beneficial effect in normotensive subjects, with no such effect in hypertensive patients. Finally, a non significant trend for a negative association between vitamin E intake and FMD was seen in the hypertensive group.

As mentioned before, hypertension has been associated with endothelial dysfunction and increased arterial stiffness ^{7, 8, 18}. In conditions such as hypertension, oxidative stress, defined as an excessive amount of oxidative substances, relative to endogenous antioxidant capacity, has shown to be increased resulting in a reduced NO-availability in these subjects ^{6, 19}. It can be speculated that in especially hypertensive patients vitamin antioxidants in regular food are not able to counteract on the increase in augmentation index and endothelial dysfunction. Antioxidants have shown to provide vascular defense against oxidative stress by reducing free radicals and protecting NO from inactivation, therefore exerting its' beneficial effects on vascular function and structure. Indeed, in a randomized control cross-over study we found that supplementation with combined vitamin C (1g) and vitamin E (400IU) improved both endothelial function and arterial stiffness in untreated essential hypertensive patients

(accepted for publication). Most likely the levels of regular food intake of vitamin C and E are too low to exert a beneficial effect on augmentation index and endothelial function, whereas combined supplementation with higher doses does have an effect. This despite the fact that the reported dietary intakes are in agreement with those commonly consumed in a general Italian population.

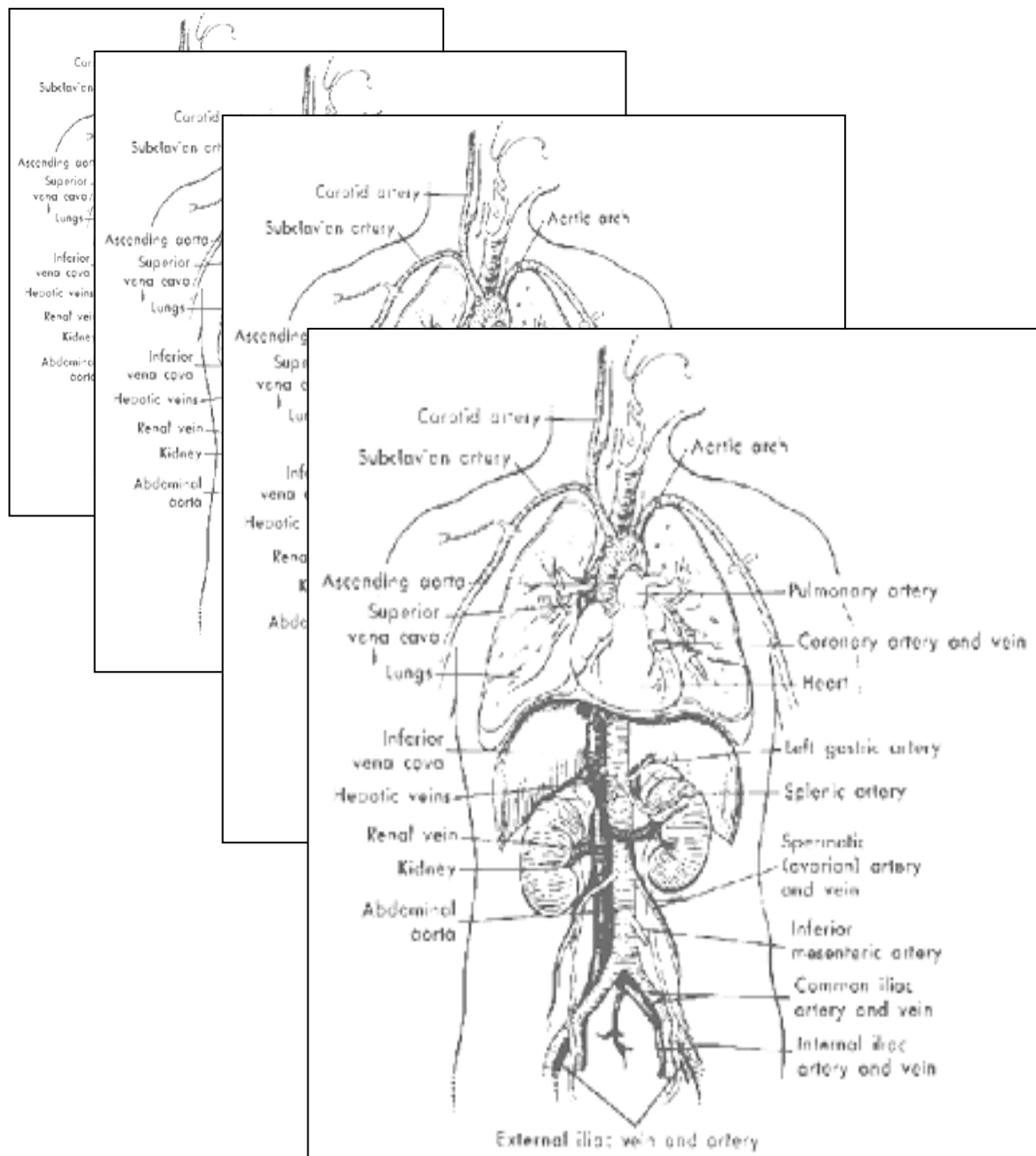
One possible limitation of the present study is the fact that no measurements of plasma levels of vitamin C and E were performed. Since there could be a discrepancy between reported intake and plasma concentrations of antioxidant vitamins ²⁰, our conclusions on the associations between intake of vitamin C and E and vascular parameters can not be generalized for plasma concentrations of vitamins.

In conclusion, this cross-sectional study demonstrated that no association exists between regular food vitamin C and E intake, augmentation index and endothelial function in hypertensive patients and normotensive subjects.

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Chapter 6

SUPPLEMENTATION WITH VITAMIN C AND E IMPROVES ARTERIAL STIFFNESS AND ENDOTHELIAL FUNCTION IN ESSENTIAL HYPERTENSIVE PATIENTS

Abstract

Background. Essential hypertension is characterized by endothelial dysfunction, arterial stiffness and increased oxidative stress. We evaluated the effect of short-term combined treatment with the antioxidants vitamin C and E on endothelial function, arterial stiffness and oxidative stress in untreated essential hypertensive patients.

Methods. A randomised, double blind, placebo controlled, crossover study design was used to assign 30, male, essential hypertensive patients to either vitamin C (1g) and E (400 IU) or placebo for 8 weeks. Endothelium-dependent response was assessed as flow mediated dilation (FMD) of the brachial artery. Arterial stiffness was assessed as central pulse wave velocity and augmentation index. Plasma markers of oxidative stress and antioxidant status were measured.

Results. After vitamin supplementation, FMD significantly improved. Central PWV was significantly reduced, while AIx tended to decrease. Plasma vitamin levels and antioxidant capacity increased significantly. Levels of oxidative stress decreased. Changes in central PWV were related with changes in levels of oxidative stress.

Conclusions. Combined treatment with Vitamin C and E has beneficial effects on endothelium-dependent vasodilation and arterial stiffness in untreated essential hypertensive patients. This effect was associated with changes in plasma markers of oxidative stress.

Introduction

Essential hypertension is an important risk factor for cardiovascular disease ¹. Most of the cardiovascular complications associated with hypertension are caused by alterations in vascular structure and function. Indeed, hypertension is associated with endothelial dysfunction and increased arterial stiffness ²⁻⁴. Both endothelial dysfunction ⁵ and arterial stiffness ⁴ are increasingly being recognized as independent cardiovascular risk factors.

Oxidative stress, defined as an excessive amount of oxidative substances, relative to endogenous antioxidant capacity, has shown to be increased in conditions such as hypertension, resulting in a reduced NO-availability in these subjects ^{6, 7}. It is hypothesized that antioxidants provide vascular defense against oxidative stress by reducing free radicals and protecting NO from inactivation, therefore exerting its' beneficial effects on vascular function and structure.

Data already indicate that acute oral administration of vitamin C (2 g) can have beneficial effects on arterial stiffness measured as augmentation index in healthy subjects ⁸. In hypertensive patients, intra-arterial administration of vitamin C has shown to improve endothelium-dependent vasodilation in the forearm microcirculation of hypertensive patients, primarily by restoring NO-availability ⁶. However, it was shown that this beneficial effect in hypertensive patients was only seen at supra-physiological doses of ascorbic acid ^{6, 9}. Indeed, available results demonstrate that prolonged oral administration of ascorbic acid at 500 mg/day for one month did not improve endothelial function in essential hypertensive patients ¹⁰. Moreover, administration with vitamin E as the sole antioxidant did not improve arterial endothelial function in older adults ¹¹.

Different studies assessing the effect of antioxidant supplementation have combined vitamin C with vitamin E, a lipid soluble vitamin with a protective effect on lipid peroxidation ^{12, 13}. This combination is of special interest, since it is known that vitamin E can have pro-oxidant properties and appropriate concentrations of vitamin C are necessary for the regeneration of vitamin E ¹⁴. Because of synergism between vitamin C and E, it is conceivable that combined supplementation with both vitamins exerts a major antioxidant effect.

At the present time no data is available on the possible effects of combined vitamin C and E on arterial stiffness and endothelial function in a population of untreated hypertensive

patients. Therefore, the aim of this study was to evaluate the effect of short-term combined antioxidant treatment with vitamin C and E on these vascular parameters and oxidative stress in untreated essential hypertensive patients.

Methods

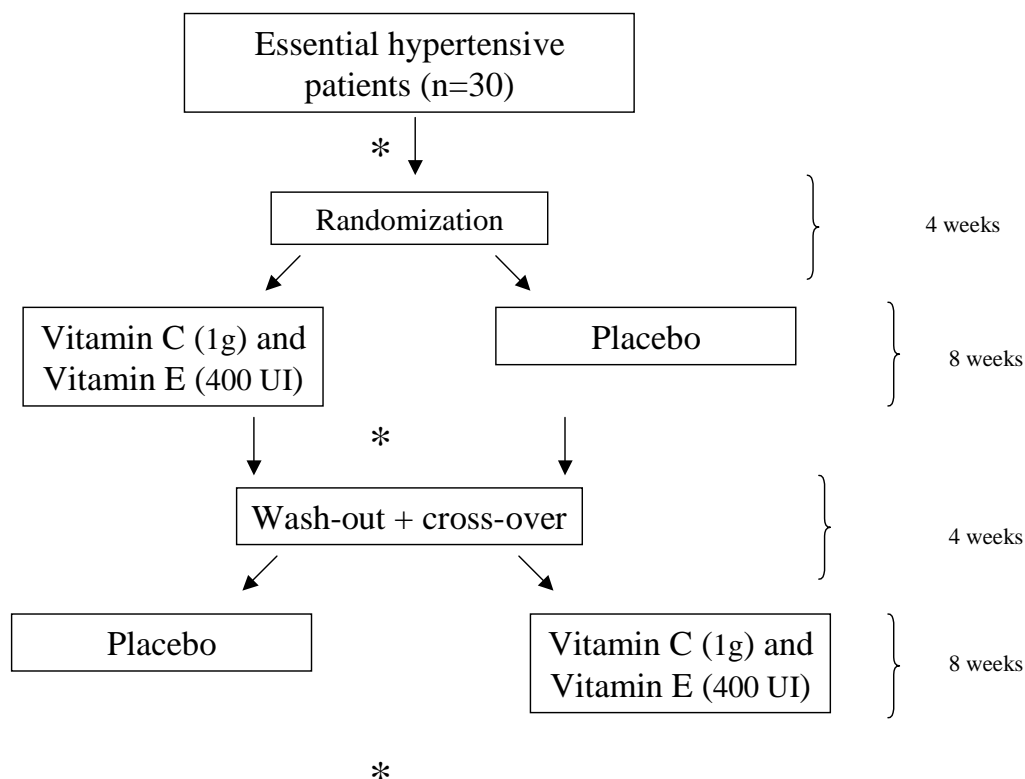
Study population

The study included 30 never treated, male, essential hypertensive patients (mean age 50 years; range 42-60 years). Patients were recruited among newly diagnosed cases in our outpatient clinic. Inclusion criteria were age between 40 and 60 years, sitting clinical blood pressure (after 10 minutes of rest) values between 140-90 and 160-99 mmHg, confirmed in two separate occasions within one month and presence of target organ damage according to ESH-ESC guidelines ¹. Exclusion criteria were history of cardiovascular disease, diabetes mellitus, dyslipidemia, BMI more than 30 kg/m², alcohol consumption (> 50 g/ day), smoking, currently performing vigorous physical exercise or using mineral or vitamin supplements, antihypertensive drugs or statins.

Study design

According to a randomized, double blind, placebo-controlled, crossover study design, patients received combined vitamin C (1g) and vitamin E (400 IU) or placebo, once a day, for 8 weeks. After a wash-out period of four weeks, patients were assigned to the inverse treatment (fig. 1). At baseline, after 8 weeks and at the end of the study, vascular studies were performed twice, on two consecutive days. In between, subjects underwent 24-hour blood pressure monitoring (ABPM) to control for blood pressure changes. Blood samples were collected for the determination of lipid and glucose profile, plasma markers of oxidative stress and antioxidant status. Patients were asked not to change physical activity habits and dietary intake during the study. Dietary intake of foods and nutrients was assessed at baseline, after 8-weeks and at the end of the study by a 3-day food record. Physical activity levels were assessed and controlled with a modified Baecke questionnaire ¹⁵. The investigation conformed with the principles outlined in the Declaration of Helsinki ¹⁶. All study participants gave written informed consent for the study.

Figure 1 - Outline of study design. * Vascular studies, ABPM monitoring, blood samples for plasma markers of oxidative stress and antioxidant status were performed.



Vascular function

After an overnight fast, measurements were performed with the subjects in supine position in a quiet, air-conditioned room (22-24°C). Radial tonometry was performed by one trained operator (Y.P.). Blood pressure was calculated as the mean value of three measurements made at 3 minutes interval by automatic device at the dominant arm (OMRON-950 CP) to calibrate the measurements. A hand held probe was placed on the right radial artery from the wrist of the dominant arm and 10-15 subsequent images were recorded. Pulse Wave Analysis (PWA) (SphygmoCor, AtCor Medical, Sydney, Australia) was used to transform the radial pressure waveform into the aortic pressure waveform by using a validated transfer function¹⁷. Three successive measurements were recorded. Augmented pressure (AP) was calculated as the difference between the first systolic peak and the second systolic peak and augmentation index (AIx) was calculated as the ratio between AP and pulse pressure (PP). Since AIx in this

study correlated with heart rate, values were normalized at a heart rate of 75 beats/min. Central pulse wave velocity (CPWV) was assessed with the same device, recording waveforms at the femoral and carotid site sequentially. Surface distance between the two recording sites was measured. A simultaneously recorded ECG was used as a reference frame to calculate wave transit time. An intra-observer reproducibility study in our lab showed a coefficient of variation of 14% for AIx and 13% for CPWV.

Endothelium-dependent response was assessed as dilation of the brachial artery to increased flow (flow mediated dilation, FMD), as previously described¹⁸. Briefly, a B-mode scan of the right brachial artery was obtained in longitudinal section between 5 cm and 10 cm above the elbow using a 7.5 MHz linear array transducer, held by a stereotactic clamp to ensure a constant image. B-mode images were triggered to the ECG signal to obtain only end-diastolic frames. Arterial flow velocity was obtained by pulsed Doppler signal at 70° to the vessel with the range gate (1.5 mm) in the center of the artery. A cuff was placed around the forearm just below the elbow and was inflated for five minutes at 250 mmHg and then deflated to induce reactive hyperemia. Endothelium-independent dilation was obtained by administration of a low dose (25 µg) of sublingual glyceril trinitrate (GTN).

Brachial artery diameter (BAD) measurements were performed after studying the acquired frames by the computerized edge detection system¹⁹. Baseline vessel size was considered as the mean of measures obtained during the first minute. FMD and response to GTN were calculated as the maximal percent increase in diameter above baseline. Doppler flow velocity was measured at baseline and within 15 seconds after cuff release. Volume blood flow was calculated by multiplying Doppler flow velocity (corrected for the angle) by heart rate and vessel cross-sectional area (πr^2). Reactive hyperemia (RH) was the maximum percent increase in flow after cuff release as compared to baseline flow.

Blood sampling and biochemical measurements

Venous blood was collected in lithium-heparin or EDTA tubes and immediately placed on ice. Plasma was immediately centrifuged and stored at -70°C until assayed. Total serum cholesterol, triglycerides, HDL-cholesterol and plasma glucose were assessed by enzymatic methods (Roche, Diagnostic, Mannheim, Germany). LDL-cholesterol was calculated with Friedewalds' equation. Oxidative stress was evaluated through measurement of plasma malondialdehyde (MDA) by spectrophotometric assay²⁰ and plasma lipoperoxides (LOOH)

with a colorimetric method, as previously described¹⁸. Antioxidant capacity was measured as plasma total antioxidant capability by measuring ferric-reducing antioxidant power (FRAP; spectrophotometric assay)²¹.

For the detection of Vitamin C, tubes were incubated for 10 minutes at 2-8° C and then centrifuged at 10.000 rpm for 10 minutes. For Vitamin E, blood samples were centrifuged for 5 minutes at 2000 rpm. Then plasma was stored at -20° C and protected from light until analyzed. High performance liquid chromatography (HPLC) was used to determine plasma vitamin C (HPLC-Analytik, Immundiagnostik AG, Bensheim, Germany) and vitamin E (Chromsystems, Instruments & Chemicals GmbH, Munchen, Germany)²².

Statistical analysis.

Data are expressed as means \pm SD, unless otherwise stated and were analyzed as change from baseline by using Student's *t*-tests for paired data or non-parametric test if necessary. Differences were considered statistically significant when $p < 0.05$. Univariate and multivariate analysis were used to examine associations between variables. All statistical procedures were performed using the Statview program (Abacus Concepts, Inc., SAS Institute, Cary, NC). Vascular responses (AIx, CPVW, FMD, response to GTN) were calculated as the mean value of the measures obtained in the two following days.

Results

During the study, dietary and physical activity habits and body weight were not modified. Clinical characteristics were similar at each phase of the study (table 1).

ABPM values were not significantly changed during the study as well as central blood pressure levels measured by PWA. After antioxidant supplementation, FMD significantly ($p < 0.001$) improved as compared to placebo, while response to GTN was not modified (fig. 2).

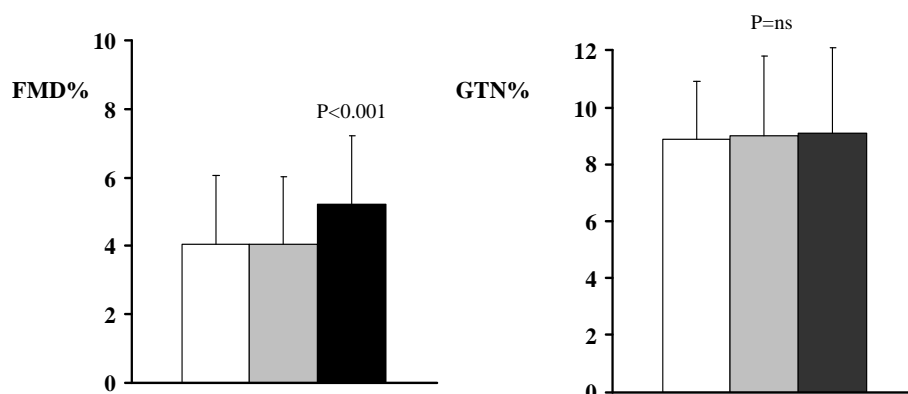
Table 1- Clinical characteristics of study population

Variable	Baseline	Vitamin C and E	Placebo
BMI (kg/m ²)	27.2±2.2	27.1±2.3	27.3±2.3
24 h-SBP (mmHg)	135±10	134±10	134±10
24 h-DBP (mmHg)	87±7	86±7	87±6
Total cholesterol (mmol/L)	5.38±0.96	5.47±0.93	5.34±0.98
HDL cholesterol (mmol/L)	1.27±0.37	1.31±0.31	1.26±0.37
LDL cholesterol (mmol/L)	3.38±0.96	3.48±0.96	3.42±0.95
Triglycerides (mmol/L)	1.53±0.6	1.56±0.7	1.53±0.6
Plasma glucose (mmol/L)	5.39±0.5	5.41±0.6	5.37±0.5
Hematocrit (%)	42.3±4.8	43.6±3.5	44.6±3.5
Hemoglobin (g/L)	159±12	157±10	156±13
Central SBP (mmHg)	139±13	139±15	138±13
Central DBP (mmHg)	90±5	89±7	89±7

BMI = body mass index; 24 h- = 24 hours ambulatory; SBP = systolic blood pressure;

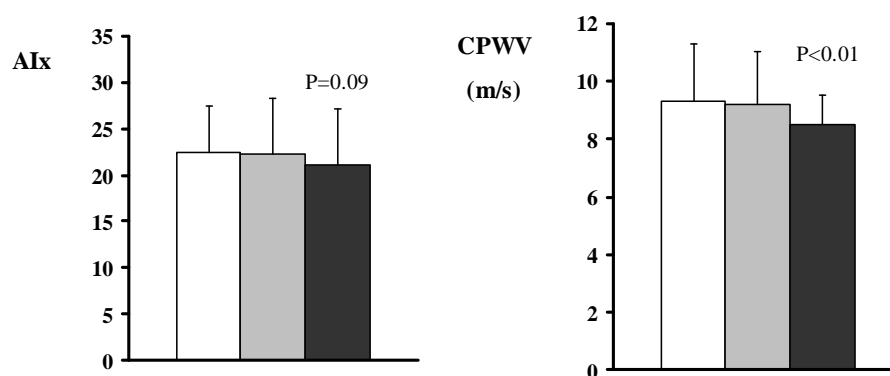
DBP = diastolic blood pressure; HDL= high-density lipoprotein; LDL= low-density lipoprotein.

Figure 2 - Differences in flow mediated dilation (FMD) and response to sublingual glyceril trinitrate (GTN) at baseline (white bars), after 8-week vitamin antioxidant supplementation (black bars) as compared to placebo (gray bars). * Denotes a significant difference (p<0.001)



After antioxidant supplementation, the outgoing pressure wave (P1) increased, but not significantly (from 33.5 to 34.3 mmHg; $p=ns$), time to first systolic peak (T1) was not modified, whereas time to the second systolic peak (T2) increased significantly (from 234 to 241 ms; $p<0.01$). AIx was reduced, although not significantly ($p=0.09$), while CPWV was significantly ($p<0.01$) lowered after antioxidant supplementation (fig. 3).

Figure 3 - Differences in augmentation index (AIx) and central pulse wave velocity (CPWV) at baseline (white bars), after 8-week vitamin antioxidant supplementation (black bars) as compared to placebo (gray bars). * Denotes a significant difference ($p<0.01$)



Changes in markers of oxidative stress and antioxidant status are reported in table 2. Compared to the placebo group, plasma vitamin C, E and FRAP levels increased significantly. After antioxidant supplementation, plasma levels of MDA decreased, although not significantly, while levels of LOOH were significantly reduced as compared to placebo. The increase in levels of vitamin C was significantly related with the changes in FRAP and LOOH ($R=0.37$; $p<0.05$, $R=-0.40$, $p<0.05$, respectively). Changes in FRAP were related with changes in MDA ($R=-0.37$, $p<0.05$).

Table 2- Markers of oxidative stress and antioxidant status

Variable	Baseline	Vitamin C and E	Placebo
MDA ($\mu\text{mol/l}$)	2.33 \pm 0.84	2.03 \pm 0.97	2.35 \pm 0.92
LOOH ($\mu\text{mol/l}$)	3.12 \pm 2.6	2.06 \pm 1.7 * †	3.10 \pm 2.3
FRAP ($\mu\text{mol/l}$)	789 \pm 141	831 \pm 139 †	783 \pm 137
Plasma Vitamin C ($\mu\text{mol/l}$)	39.12 \pm 22.43	52.35 \pm 32.65 * †	33.95 \pm 22.83
Plasma Vitamin E ($\mu\text{mol/l}$)	20.41 \pm 8.68	25.50 \pm 9.36 †	19.81 \pm 7.55

MDA: malondialdehyde; LOOH: lipoperoxides; FRAP: Ferric reducing ability of Plasma; * $P < 0.05$ vs. baseline, † $P < 0.05$ vs. placebo.

At baseline, FMD was related with BAD ($R = -0.47$; $p < 0.01$), levels of vitamin C ($R = 0.40$; $p < 0.05$) and FRAP ($R = 0.36$; $p < 0.05$). AIx correlated significantly with age ($R = 0.50$; $p < 0.01$) and inversely with FRAP ($R = -0.42$; $p < 0.05$). CPWV was related to SBP ($R = 0.40$; $p = 0.03$), age ($R = 0.38$; $p < 0.05$), LOOH ($R = 0.38$; $p < 0.05$) and levels of MDA ($R = 0.39$; $p < 0.05$). Multivariate analysis including also other possible confounders (age, blood pressure values, LDL and HDL cholesterol) showed that for FMD only BAD ($p = 0.02$) remained a significant variable ($R^2 = 0.44$). For AIx, age ($p < 0.01$) resulted as a significant predictor ($R^2 = 0.32$) and SBP ($p = 0.03$) for CPWV ($R^2 = 0.40$).

Changes in FMD after antioxidant supplementation were not related with changes in CPWV ($R = -0.14$; $p = \text{n.s.}$). Changes in FMD showed a negative, but not significant correlation with changes in MDA ($R = -0.31$, $p = 0.07$). Changes in CPWV after antioxidant supplementation, were related with changes in levels of MDA ($R = 0.43$; $p < 0.05$) and borderline with changes in levels of FRAP ($R = -0.30$, $p = 0.06$). The changes in AIx were significantly related with the observed differences in levels of MDA ($R = 0.41$; $p < 0.05$). When multivariate analysis was performed including possible confounding variables including age, blood pressure values, LDL and HDL cholesterol, changes in MDA remained significantly related with changes in CPWV ($R^2 = 0.31$) and AIx ($R^2 = 0.29$).

Discussion

The present study demonstrates that oral supplementation with vitamin C and E for 8 weeks improves arterial stiffness and endothelial function in untreated essential hypertensive patients. Supplementation with vitamins increased total plasma antioxidant capacity, vitamin

C and vitamin E levels and reduced the plasma oxidative markers LOOH and MDA, indicating a beneficial modulation of systemic markers of oxidative stress.

To our knowledge no previous study has investigated the effect of antioxidant vitamins on both arterial stiffness and endothelial function in essential hypertensive patients. Concerning arterial stiffness data are not consistent, showing either a beneficial^{8, 23} or no effect²⁴ of different kinds of antioxidant supplementation on parameters of arterial stiffness in apparently healthy subjects. However, an improvement in central aortic AIx was shown after 500 mg/day oral vitamin C supplementation for one month in a population of type II diabetes²⁵.

In the present study, oral supplementation with combined vitamins significantly improved in CPWV. Importantly, the improvement in CPWV in the present study was related with a decreased level of oxidative stress markers. Furthermore, AIx, an integrated index of arterial stiffness and arterial wave reflection, was lowered after antioxidant supplementation, but not significantly. Since central blood pressure levels remained unchanged, the change in AIx can be most likely explained by the observed increase of the outgoing pressure wave (P1). A significant increase in T2 was also observed. Thus, supplementation with antioxidants seems to have a greater beneficial effect on central large artery function as demonstrated with the results obtained with CPWV. These results suggest that oxidative stress plays a role in determining arterial stiffness in untreated hypertensive patients. Since an 8-week treatment is not sufficient to induce any significant change in vascular structure, it is conceivable that functional mechanisms account for the improvement in arterial stiffness, such as an improvement in endothelial function²⁶ and a possible beneficial effect of antioxidants on protein cross-linking in conduit arteries, including the aorta²⁷.

FMD improved after antioxidant supplementation, while no significant changes were observed in the response to GTN. These results indicate that treatment with combined vitamins selectively improves endothelium-dependent vasodilation of the brachial artery. The improvement in FMD was not significantly related to changes in antioxidant status or oxidative stress after vitamin supplementation. However, it was already suggested that evaluation of plasma markers of oxidative stress may not accurately reflect oxidative status at the level of endothelial cells¹⁸. Previous studies have shown that the beneficial effect of ascorbic acid administration on endothelial function in hypertensive patients was only achieved at supra-physiological doses by intra-arterial administration^{6,9}, while prolonged oral

administration (500 mg/day for one month) was not effective¹⁰. On the contrary, a beneficial effect of a similar treatment with ascorbic acid has been described in patients with CAD²⁸. Finally, in apparently healthy older subjects, 10 weeks daily supplementation with vitamin E (1000 IU/day) failed to show a beneficial effect on endothelial function¹¹.

It is conceivable that the beneficial effect of the chronic oral antioxidant treatment on endothelial function, at least in patients at relatively low cardiovascular risk such as our population of untreated hypertensive patients, requires the administration of combined Vitamin C and Vitamin E at appropriate dosages. Indeed, vitamin C can reverse the pro-oxidant status of Vitamin E in turning α -tocopheroxyl back into α -tocopherol¹⁴. Thus, combined treatment can more effectively counteract on the NO scavenging action of oxidative stress and can also increase NO production *persé*²⁹. In fact, ascorbic acid can improve tetrahydrobiopterin availability in the vasculature and α -tocopherol exerts a direct stimulatory effect on e-NOS activation, an effect which is amplified by ascorbic acid, suggesting that both compounds may act synergistically in optimizing endothelial NO synthesis²⁹.

Our results may have clinical implications since both endothelial dysfunction and arterial stiffness are early mechanisms of vascular alterations and independent predictors of cardiovascular diseases^{4, 5}. The major part of controlled studies evaluating the effect of vitamin supplementation on vascular lesions and cardiovascular events, show inconclusive results³⁰⁻³³. One of the possible explanations could reside in the clinical conditions of the study populations in which the presence of advanced vascular alterations or associated clinical conditions might influence negatively on the beneficial effect of antioxidant vitamins. An alternate explanation could be the long term use of vitamin E (α tocopherol) as the sole antioxidant supplement in some of the studies^{11, 31, 32, 34}. Thus our results may suggest that an early combined treatment with vitamin C and E in uncomplicated essential hypertensive patients can have beneficial effects in the prevention of vascular alterations and cardiovascular events.

In our study clinic, ambulatory and central blood pressure levels were not changed by antioxidant vitamin supplementation. Although one study suggested that treatment with oral vitamin C (500 mg/day, 30 days) was associated with a reduction in blood pressure values³⁵, a recent review revealed that no real evidence exists for an effect of antioxidant supplementation in preventing or treating high blood pressure³⁶.

One possible limitation of the present study is the fact that no measurements of intravascular oxidative stress were performed. Therefore, our conclusions on the associations between differences in oxidative stress and vascular parameters can be over- or under estimated. Furthermore, the results of the present study do not permit to draw conclusions on an in vivo interaction of the combined supplementation, since the effects of Vitamin C or E alone were not evaluated.

In conclusion, this randomized study demonstrated that as compared to placebo combined oral antioxidant treatment with vitamin C and E for 8 weeks has beneficial effects on arterial stiffness and endothelial function in untreated essential hypertensive patients. The improvements in arterial stiffness and possibly in endothelial function seem to be due to an effective increase in antioxidant defenses leading to a subsequent decrease in oxidative stress. Further research is still needed to study the potential long-term benefit of vitamin antioxidant supplementation on prevention of early vascular alterations.

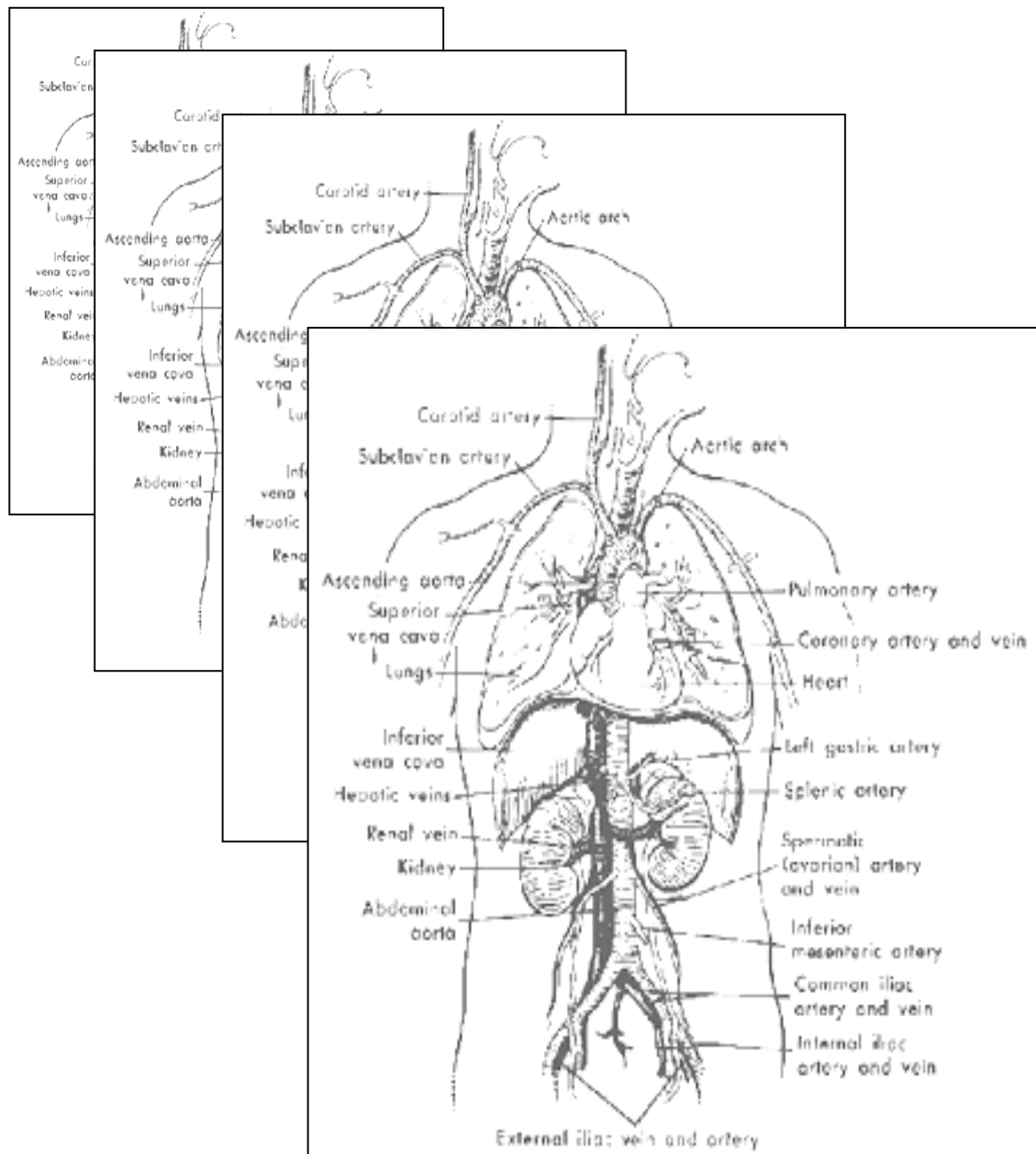
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Chapter 7

RELATIONSHIP BETWEEN MEASUREMENTS OF PULSE WAVE ANALYSIS, PULSE WAVE VELOCITY AND FLOW MEDIATED DILATION IN NORMOTENSIVE AND HYPERTENSIVE SUBJECTS (preliminary results)

Introduction

Endothelial dysfunction ¹ and increased arterial stiffness ² are increasingly being recognized as powerful predictors of cardiovascular disease morbidity and mortality. Both conditions have shown to occur simultaneously in populations at high risk for cardiovascular disease such as patients with hypercholesterolemia, hypertension and diabetes.

The endothelium has important properties in controlling both vascular tone and vascular structure by the production of multiple relaxing factors such as nitric oxide, prostacyclin and a not yet identified hyperpolarizing relaxing factor (EDHF) ³. Furthermore, endothelial dysfunction is an early, functional and potentially reversible alteration of endothelial cells, resulting from an impairment in NO availability ⁴ and can be detected by flow mediated dilation of the brachial artery ⁵.

Arterial stiffening, a result of structural and functional alterations of the vascular wall can be measured by means of pulse wave velocity (PWV) and pulse wave analysis (PWA) ⁶. Lately, it has been suggested that nitric oxide may play a role in regulating arterial stiffness ⁷. Studies have shown that substances associated with increase in NO production, including glyceryl trinitrate (GTN), reduce arterial stiffness and wave augmentation, whereas inhibitors of NO, such as L-NMMA, increase indexes of arterial stiffness ⁷. Since endothelial dysfunction and arterial stiffness both play a role in the atherosclerotic process, studies have already suggested that measurements of endothelial function and arterial stiffness are correlated. These studies however are not conclusive and are conducted in different populations. The aim of this study was to evaluate the possible relationship between endothelial function measured as flow mediated dilation (FMD) of the brachial artery and arterial stiffness assessed with PWA and PWV in a large population of untreated essential hypertensive patients and normotensive controls.

Methods

Study population

In this cross-sectional study, 242 (79 females) hypertensive patients (HT) between 20 and 70 years of age were recruited for the study from February 2004 to December 2005. To serve as control, 192 normotensive subjects (NT) (64 females) were added. Patients with essential

hypertension were recruited from the newly diagnosed cases in our outpatient clinic and were enrolled if sitting clinic arterial blood pressure (after 10 minutes of rest) was consistently found to be greater than 140/90 mmHg, confirmed in two separate occasions within one month according to European guidelines⁸. Patients had never been treated (n=172) or had a history of recent or discontinuous treatment (n=70), with a pharmacological washout for at least 3 weeks before entering the study. Subjects with medical history of cardiovascular events, cardiovascular diseases or other diseases requiring medical treatment, were excluded for the study. Subjects were defined as normal according to the absence of familial history of essential hypertension and blood pressure values below 140/90 mmHg.

FMD, augmentation index (AIx) and radial-carotid pulse wave velocity (BPWV) were measured in all subjects. Carotid femoral pulse wave velocity (CFPWV) was assessed in a subgroup of 112 subjects (HT=66; NT=46). Body weight and height were measured in all subjects. Body mass index (BMI) was calculated as weight/height². Blood samples were collected following an overnight fast to measure lipid and glucose profile. Information on current smoking status (smoker/non smoker) was also collected. The study complied with the declaration of Helsinki of the World Medical Association and the local ethical committee approved the protocol. All study participants gave written informed consent for the study.

Vascular function

After an overnight fast, measurements were performed with the subjects in supine position in a quiet, air-conditioned room (22-24°C). Radial tonometry was performed by one trained operator (Y.P.). Blood pressure was calculated as the mean value of three measurements made at 3 minutes interval by automatic device at the dominant arm (OMRON-950 CP) to calibrate the measurements. A hand held probe was placed on the right radial artery from the wrist of the dominant arm and 10-15 subsequent images were recorded. PWA (SphygmoCor, AtCor Medical, Sydney, Australia) was used to transform the radial pressure waveform into the aortic pressure waveform by using a validated transfer function⁹. Three successive measurements were recorded. Augmented pressure (AP) was calculated as the difference between the second systolic peak and first systolic peak and AIx was calculated as the ratio between AP and pulse pressure (PP). Since AIx in this study correlated with heart rate, values were normalized at a heart rate of 75 beats/min.

CFPWV was assessed with the same device, recording waveforms at the femoral and carotid site sequentially. BPWV was assessed recording waveforms at the radial and carotid site. Surface distance between the two recording sites was measured. A simultaneously recorded ECG was used as a reference frame to calculate wave transit time. An intra-observer reproducibility study in our lab showed a coefficient of variation of 14% for AIx and 13% for CFPWV and BPWV.

Endothelium-dependent response was assessed as dilation of the brachial artery to increased flow (FMD), as previously described ¹⁰. Briefly, a B-mode scan of the right brachial artery was obtained in longitudinal section between 5 cm and 10 cm above the elbow using a 7.5 MHz linear array transducer, held by a stereotactic clamp to ensure a constant image. B-mode images were triggered to the ECG signal to obtain only end-diastolic frames. Arterial flow velocity was obtained by pulsed Doppler signal at 70° to the vessel with the range gate (1.5 mm) in the center of the artery. A cuff was placed around the forearm just below the elbow and was inflated for five minutes at 250 mmHg and then deflated to induce reactive hyperemia. Endothelium-independent dilation was obtained by administration of a low dose (25 µg) of sublingual glyceril trinitrate (GTN).

Brachial artery diameter measurements were performed after studying the acquired frames by the computerized edge detection system ¹¹. Baseline vessel size was considered as the mean of measures obtained during the first minute. FMD and response to GTN were calculated as the maximal percent increase in diameter above baseline. Doppler flow velocity was measured at baseline and within 15 seconds after cuff release. Volume blood flow was calculated by multiplying Doppler flow velocity (corrected for the angle) by heart rate and vessel cross-sectional area (πr^2). Reactive hyperemia (RH) was the maximum percent increase in flow after cuff release as compared to baseline flow.

Statistical analysis.

Simple regression analysis was used to examine associations between variables. Multiple linear regression analysis was performed in the whole study group and separately in HT patients and NT subjects, including in the final models all variables that in the preliminary regression analysis were related to the vascular parameters. Differences were considered statistically significant when $p < 0.05$. All statistical procedures were performed using the Statview program (Abacus Concepts, Inc., SAS Institute, Cary, NC).

Preliminary results

No significant differences were found in age, gender distribution, BMI, smoking, total cholesterol, HDL-cholesterol or plasma glucose levels between HT and NT. HT patients showed significantly ($p<0.0001$) lower FMD (5.1 ± 2.7) as compared with normotensive controls (6.0 ± 2.7), while response to GTN was similar. AIx resulted significantly higher in HT (25.7 ± 11.6) as compared to NT (19.5 ± 12.2). CFPWV was higher in HT (8.8 ± 1.9 m/s) as compared to controls (8.2 ± 1.9 m/s) as well as BPWV (9.5 ± 1.4 m/s vs. 8.7 ± 1.1 , respectively) and central pulse pressure (cPP) (50.1 ± 12.6 mmHg vs. 41.3 ± 8.4 mmHg, respectively). Brachial artery diameter (BAD) was significantly higher in HT as compared to NT (4.54 ± 0.91 vs 4.26 ± 0.79 mm).

In the whole group, FMD correlated with response to GTN ($r=0.36$, $p<0.0001$), cPP ($r=-0.18$, $p<0.001$) and BPWV ($r=-0.15$, $p<0.01$), but not with CFPWV or AIx. Multiple regression analysis showed that age and BAD resulted as significant predictors of FMD ($R^2=0.24$).

AIx was associated with cPP ($r=0.42$, $p<0.0001$), CFPWV ($r=0.31$, $p<0.001$) and BPWV ($r=0.22$, $p<0.0001$), but not with response to GTN. Multiple regression analysis revealed that age, DBP, height, BAD, CFPWV and SBP resulted as significant predictors of AIx ($R^2=0.64$). Leaving CFPWV out of the model, because of lower number of subjects, age, diastolic blood pressure (DBP), BAD, height and BPWV become determinants of AIx ($R^2=0.57$).

In the whole group, BPWV was associated with FMD, AIx (both see above), CFPWV ($r=0.32$, $p<0.001$) and cPP ($r=0.20$, $p<0.0001$), but not to response to GTN. Finally, DBP and CFPWV resulted as significant predictors ($R^2=0.23$). Discarding CFPWV from the model, DBP and BAD emerge as predictors of BPWV ($R^2=0.20$).

CFPWV showed associations with cPP ($r=0.21$, $p=0.03$), BPWV, AIx, but not with FMD (see above) or response to GTN. Multiple regression analysis showed that systolic blood pressure (SBP), DBP, AIx, BPWV, BAD and cPP resulted as significant determinants of CFPWV ($R^2=0.37$).

In the HT group, FMD correlated with response to GTN ($r=0.33$, $p<0.0001$), BPWV ($r=-0.20$, $p<0.01$) and cPP ($r=-0.14$, $p=0.04$), but not with CFPWV or AIx. Multiple regression analysis showed that BAD, age, response to GTN and BPWV resulted as significant predictors of

FMD ($R^2 = 0.29$). AIX was associated with cPP ($r=0.38$, $p<0.0001$), CFPWV ($r=0.24$, $p=0.05$) and borderline with BPWV ($r=0.21$, $p=0.08$), but not with response to GTN. Multiple regression analysis revealed that age, BAD, DBP, height and CFPWV resulted as significant predictors of AIX ($R^2=0.54$). Discarding CFPWV from the model, age, height, BAD, DBP, SBP and BMI become significant determinants of AIX ($R^2=0.48$).

BPWV was associated with FMD and borderline with AIX (see above), but not with CFPWV, cPP or response to GTN. Finally, DBP and FMD resulted as significant predictors of BPWV ($R^2=0.16$). CFPWV showed associations with cPP ($r=0.25$, $p=0.05$) and AIX (see above), but not with FMD or response to GTN. AIX and BAD resulted as significant determinants of CFPWV ($R^2=0.19$).

In the normotensive group, FMD correlated with response to GTN ($r=0.39$, $p<0.0001$), but not with BPWV, CFPWV or AIX. Multiple regression analysis showed that response to GTN, BAD and age resulted as significant predictors of FMD ($R^2 = 0.26$).

AIX was associated with cPP ($r=0.38$, $p<0.0001$), CFPWV ($r=0.35$, $p=0.02$) and BPWV ($r=0.20$, $p<0.01$), but not with response to GTN. Multiple regression analysis revealed that age, height and DBP resulted as significant predictors of AIX ($R^2=0.63$). Leaving CFPWV out of the model showed that age, DBP, BAD and height result as determinants of AIX ($R^2=0.60$).

BPWV was associated with AIX (see above) and CFPWV ($r=0.54$, $p<0.0001$), but not with FMD or response to GTN. Finally, CFPWV resulted as significant predictor of BPWV ($R^2=0.34$). Without CFPWV added in the regression model, only DBP resulted as significant predictor of BPWV ($R^2=0.11$). Finally, CFPWV showed associations with BPWV and AIX (see above), but not with FMD or response to GTN. Finally, SBP and BPWV resulted as significant determinants of CFPWV ($R^2=0.52$).

Discussion

Our results reveal that brachial artery endothelial function, measured as FMD and the measurements of augmentation index and CFPWV are not associated in our population of hypertensive patients and normotensive subjects. The lack of a significant relationship between conduit artery endothelial function and central arterial stiffness and wave reflection suggests that these parameters are different entities of structure and function of the vascular

tree. However, BPWV, a marker of peripheral arterial stiffness, and FMD were related in the essential hypertensive patients. This could confirm data from studies, which showed that the measurement of FMD of the brachial artery was affected by local elastic properties in high-risk patients or the elderly ¹². It was hypothesized that a stiff artery, with preserved NO availability, cannot reach the maximal dilation due to physical restrictions ¹³. This could highly influence the validity of FMD measurements especially in high-risk patients.

However, in cardiovascular risk assessment both endothelial function and arterial stiffness both play a role in the atherosclerotic process. Both parameters have shown their value in predicting cardiovascular disease ^{1,2} and even so important they have shown to be modifiable factors. Therefore in the risk assessment and in studies evaluating vascular function and structure it is suggested to measure both endothelial function and arterial stiffness in order to have a complete and proper view of the vascular tree and to prevent possible validity problems.

Figure 1 - Correlation between FMD and AIx in hypertensive patients and normotensive subjects

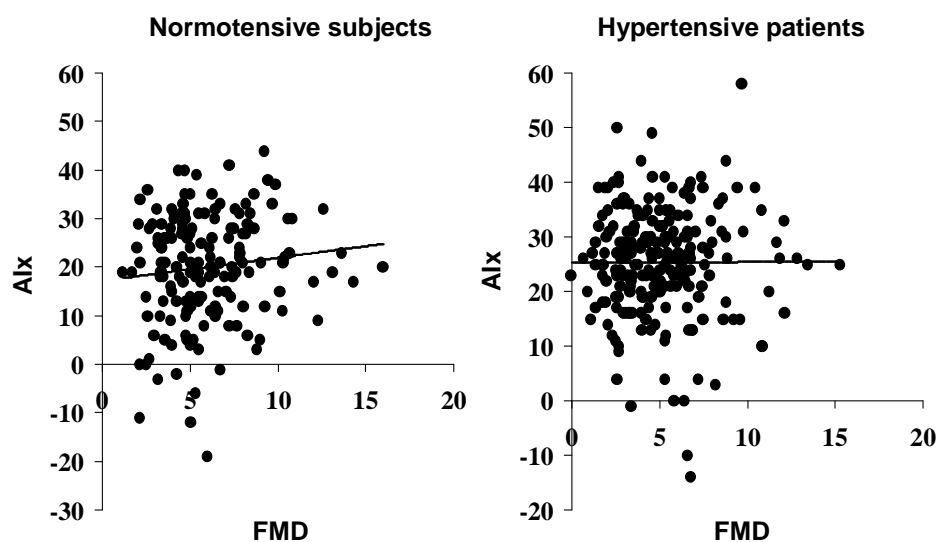
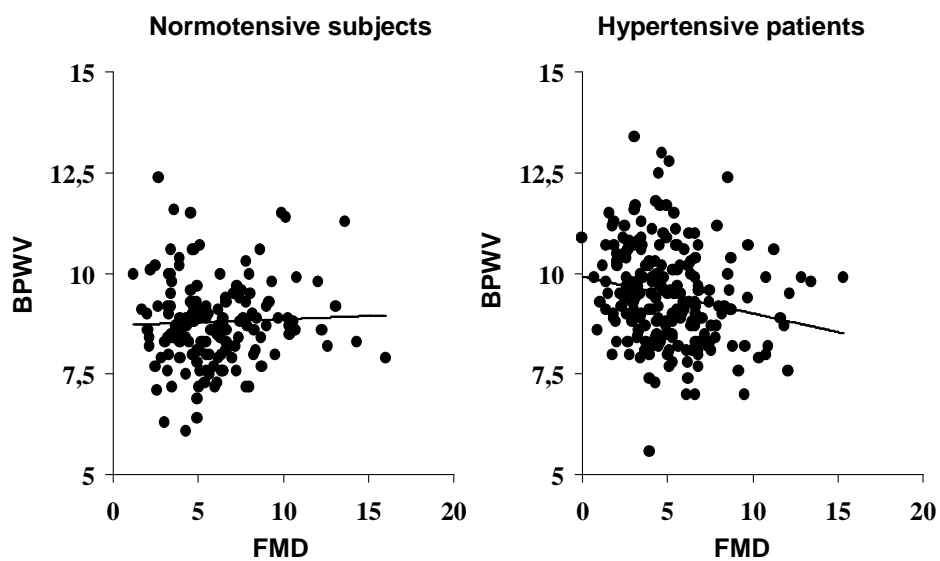


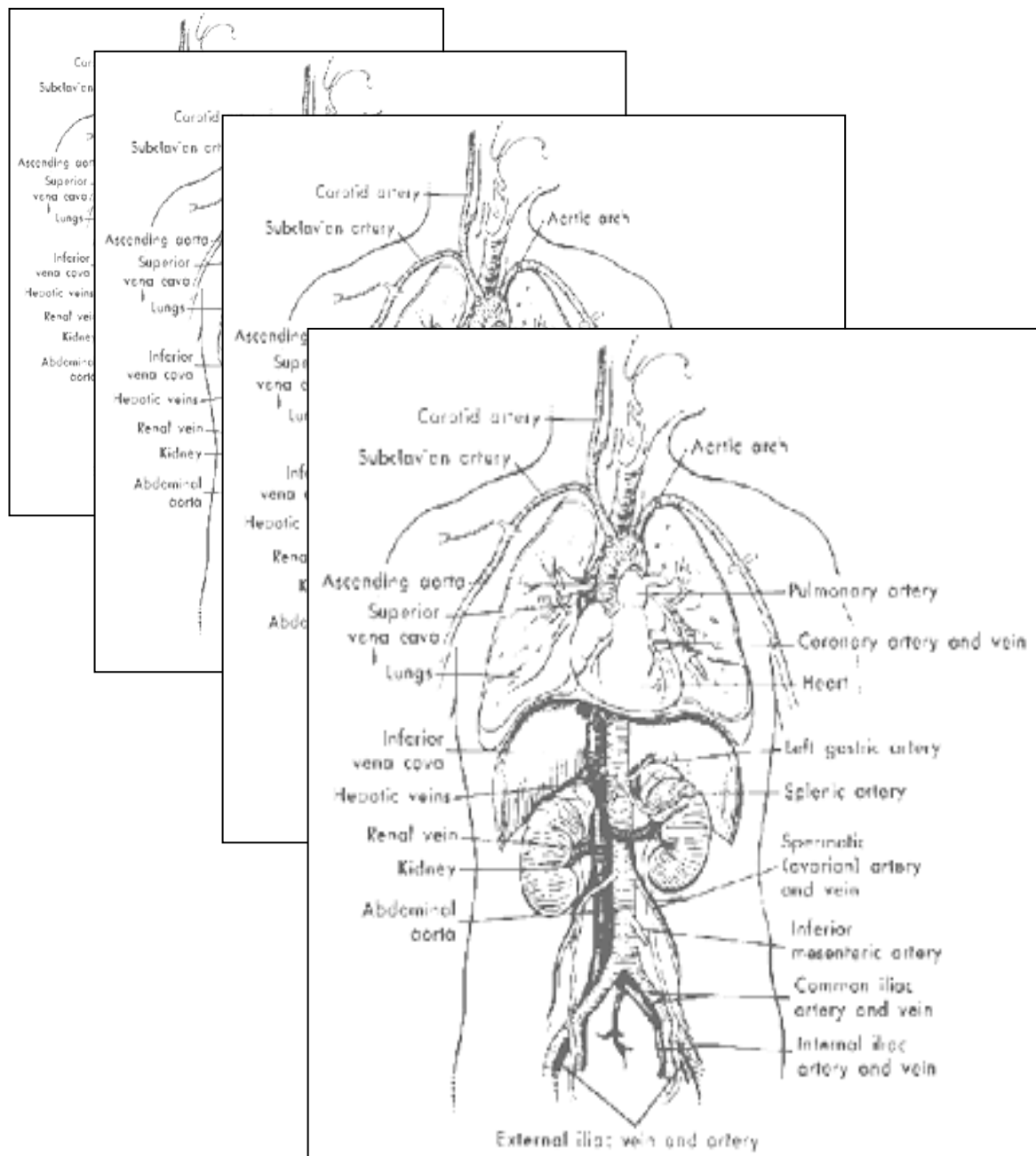
Figure 2 - Correlation between FMD and BPWV in hypertensive patients and normotensive subjects



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Chapter 8

GENERAL DISCUSSION

In the prevention and reduction of cardiovascular disease, different modifiable cardiovascular risk factors have already been identified. Hypertension is one of the major important modifiable risk factors for cardiovascular disease¹. The stratification of risk in hypertensive patients is related to blood pressure values, other cardiovascular risk factors such as unfavorable lipid profile, obesity, diabetes mellitus, target organ damage and associated clinical conditions². Patients with essential hypertension are characterized by early alterations in vascular structure and function, such as endothelial dysfunction^{3, 4} and increased arterial stiffness⁵ and wave reflection⁶ and these alterations are increasingly being recognized as independent cardiovascular risk factors^{5, 7}. Hypertension is not only a major cardiovascular risk factor but is also likely to be accompanied by other CV risk factors such as unfavorable lipid profile, insulin resistance, obesity and diabetes mellitus².

The metabolic syndrome represents the association of high blood pressure, increased abdominal obesity, dyslipidemia and altered glucose tolerance⁸ and is accompanied by an increase in CV disease in the general population, but also in essential hypertensive patients⁹. It has been suggested that the presence of the metabolic syndrome exerts a deleterious effect on vascular alterations, especially in subjects with hypertension¹⁰⁻¹². However, in **chapter 2** we showed that in our population of untreated hypertensive patients, the presence of the metabolic syndrome does not account for a ulterior increase in endothelial dysfunction or augmentation index other than that caused by the high levels of blood pressure. These results support the hypothesis that blood pressure is the most important component of the metabolic syndrome in defining conduit artery endothelial function and augmentation index, an integrated index of arterial stiffness and peripheral wave reflection, in these subjects. The lack of influence of the metabolic syndrome on the vascular parameters could be explained by the presence of overt disease such as untreated hypertension. In our population of untreated hypertensive patients, the incremental risk attributable, for example, to raised triglycerides or low HDL-cholesterol, is likely to be overwhelmed by the presence of hypertension. Furthermore, we don't know if in our population central arterial stiffness was affected since we only measured augmentation index as an index of arterial stiffness. Indeed, previous studies indicated that the metabolic syndrome can increase central arterial stiffness, measured as carotid-femoral PWV^{10, 11}. Another explanation could reside in the fact that we used the ATP III criteria⁸, which does not take insulin resistance into account as a factor of the metabolic syndrome.

A wide range of interventions are evaluated to prevent or modify the above mentioned vascular functional and structural alterations. We however focused our attention on the possible effect of lifestyle habits on these parameters. In all the research available on life style modifications and cardiovascular disease, it is already clear that being physically active exerts a beneficial effect on cardiovascular risk factors. It has been shown that regular physical activity lowers blood pressure, which results in a decrease in CHD, stroke and total mortality¹³. It has been hypothesized that the reduction in CV events could be related to the parallel improvement in vascular parameters. The beneficial effect of physical activity has been shown in the attenuation of the age related increase in endothelial dysfunction¹⁴ and different studies have shown an improvement of endothelial function with physical activity interventions¹⁵. Furthermore physical activity has shown to exert beneficial effects on arterial stiffness, since both pulse wave velocity and augmentation index have shown to be reduced by physical exercise¹⁶⁻¹⁹. However, no data was available on the effect of regular habitual physical activity on arterial wave reflection.

Therefore, in **chapter 3** we showed that regular physical activity was associated with a lower augmentation index in normotensive subjects, but this association was less clear in untreated hypertensive patients. The most likely explanation for these findings is that the moderate physical activity exerted in our population was not sufficient to show a similar effect on wave reflection in patients with or without hypertension. Since hypertension increases arterial stiffness and arterial wave reflection, it is conceivable that the degree of physical activity was less effective to counterbalance the increase in augmentation index in hypertensive patients. These results could suggest that higher levels of physical activity are required to modify arterial wave reflection in untreated hypertensive patients, at least when hypertension is not yet treated with pharmacological interventions to normalize blood pressure levels.

On the other hand, in **chapter 4** we showed that the age-related decrease in endothelial function measured as FMD is attenuated in elderly athletes, who performed endurance exercise for longer periods, as compared to sedentary peers. In sedentary individuals, increasing age was associated with a decrease in endothelial function measured as FMD, with a parallel attenuation of plasma antioxidant capacity and increased markers of oxidative stress. However, the decrease in FMD in elderly subjects who performed aerobic endurance exercise was attenuated. Moreover this effect was associated with higher plasma antioxidant capacity.

Therefore, the mechanism responsible for the beneficial effect of physical activity on vascular function could in part be due to an increased antioxidant defense capacity. Cardiovascular risk factors such as hypertension, but also (sedentary) aging are accompanied by oxidative stress, defined as an excessive amount of oxidative substances, relative to endogenous antioxidant capacity, resulting in a reduced NO-availability^{20, 21}. Therefore, an increase in antioxidant capacity could counteract on the deleterious effect of oxidative stress on the vascular tree.

Regarding the possible effects of antioxidants, studies have been done on the effect of antioxidant vitamins, in regular foods or as food supplements and the risk of cardiovascular disease. The apparent beneficial results of high intake of antioxidant vitamins in observational studies are not conclusive. We hypothesized that high dietary intake of vitamin supplementation or experimental oral supplementation with antioxidants could have beneficial effects on cardiovascular disease especially in patients with essential hypertension.

However, in **chapter 5** we showed that the intake of vitamin antioxidant C and E through regular food was not related with augmentation index or endothelial function in both hypertensive patients and normotensive subjects. These results suggest that the relatively low intake of vitamin C and E in regular food is not sufficient to have an effect on conduit artery endothelial function or arterial wave reflection.

Indeed, in **chapter 6** we were able to demonstrate that in a subgroup of patients with untreated essential hypertension, short term supplementation with combined higher doses of the antioxidant vitamins C and E had beneficial effects on both endothelial function and arterial stiffness. Interestingly, these improvements were parallel with an increase in total plasma antioxidant capacity, vitamin C and vitamin E levels and a reduction in the plasma oxidative markers, indicating a beneficial modulation of systemic markers of oxidative stress. These results confirm the central role of oxidative stress in inducing endothelial dysfunction and increased arterial stiffness, at least in patients with essential hypertension, and the necessity to use adequate doses and combination of antioxidants to obtain beneficial effects. Finally, since both endothelial function and arterial stiffness improved with antioxidant supplementation and both conditions play a role in the atherosclerotic process, it can be suggested that measurements of endothelial function and arterial stiffness are correlated. However, our results obtained in both hypertensive and normotensive subjects, described in **chapter 7**, suggest that the measurements of FMD, PWA and PWV assess different entities of

structure and function of the vascular tree. Therefore in the risk assessment and in studies evaluating vascular function we recommend to measure both endothelial function and arterial stiffness in order to have a complete and proper view of the vascular tree.

Conclusions

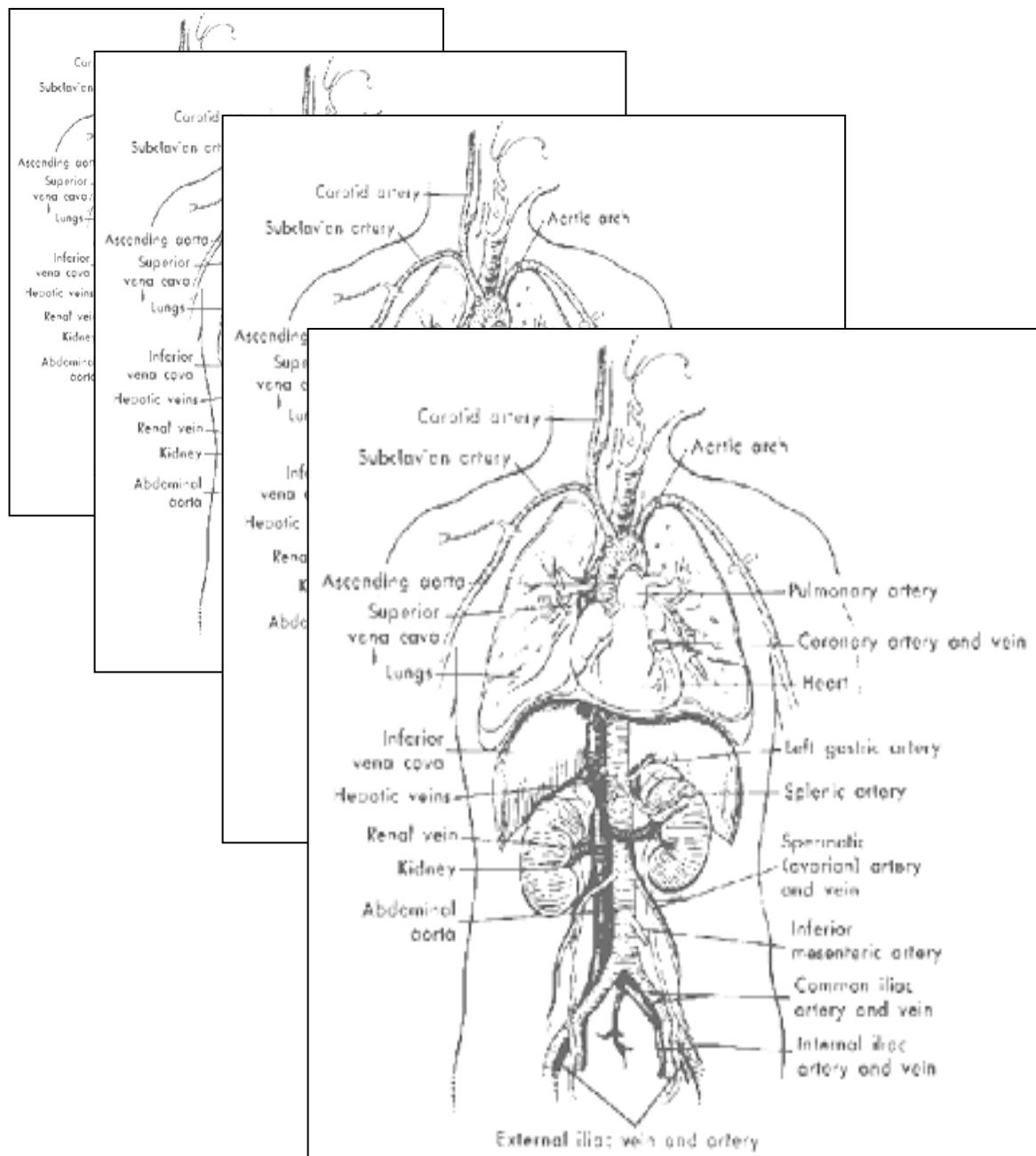
It is well established that essential hypertension is a powerful predictor of cardiovascular disease, accompanied by endothelial dysfunction and increased arterial stiffness and peripheral wave reflection. The presence of the metabolic syndrome in our patients with overt disease such as hypertension does not seem to be associated with ulterior increase in endothelial dysfunction or arterial stiffness. In our population, regular physical activity was associated with a lower augmentation index in normotensive subject and to a far lesser extent in hypertensive patients. Furthermore we found an association between regular high intensity endurance exercise, antioxidant capacity and preserved endothelial function in healthy older men, confirming the important mechanism of antioxidant defenses against oxidative stress. In this line we also showed a beneficial effect of short term, high dose combined antioxidant supplementation on arterial stiffness and endothelial function in a small group of untreated essential hypertensive patients, while this association with the vascular parameters was not observed when evaluating the effects of regular dietary antioxidant intake in the whole population. Finally, we revealed that the measurements of FMD, PWA and PWV assess different entities of structure and function of the vascular tree and it is recommended to measure both vascular parameters in future research.

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Riassunto Italiano

L'ipertensione essenziale è caratterizzata da alterazioni vascolari precoci come la disfunzione endoteliale e l'aumento della rigidità arteriosa e della riflessione dell'onda sfigmica. Tali alterazioni sono considerate predittori indipendenti dello sviluppo di eventi cardiovascolari.

Poichè l'associazione di fattori di rischio multipli, come nella sindrome metabolica, si associa ad un aumentato rischio di morbidità e mortalità cardiovascolare nei pazienti con ipertensione arteriosa, il primo scopo di questa tesi è stato quello di valutare l'effetto della presenza della sindrome metabolica sulla disfunzione endoteliale, la rigidità arteriosa e la riflessione dell'onda sfigmica in un gruppo di pazienti ipertesi essenziali.

L'attività fisica e la dieta sono le più importanti misure non farmacologiche per la prevenzione del rischio cardiovascolare. Tali misure possono avere effetti favorevoli sia sulla disfunzione endoteliale, che sulla rigidità arteriosa e la riflessione dell'onda sfigmica. Pertanto il secondo scopo della tesi è stato quello di valutare l'effetto dell'attività fisica, dell'apporto dietetico e della supplementazione di vitamine antiossidanti su tali parametri vascolari nei pazienti ipertesi essenziali.

Sono state utilizzate valutazioni trasversali ed uno studio randomizzato di intervento verso placebo, in doppio cieco con disegno di crossover. La risposta endotelio-dipendente è stata valutata come vasodilatazione mediata da incremento di flusso (flow mediated dilation) dell'arteria omerale, la rigidità arteriosa come pulse wave velocity centrale ed augmentation index. Il livello di attività fisica e di dieta abituale è stato ottenuto con questionari validati. Infine, sono stati misurati alcuni parametri plasmatici di stress ossidativo e di capacità antiossidante.

Nella nostra popolazione di pazienti ipertesi essenziali non trattati la presenza della sindrome metabolica non determinava un ulteriore peggioramento dei parametri strutturali e funzionali vascolari. La pressione arteriosa rappresentava il più importante componente della sindrome metabolica nell'influenzare la funzione endoteliale e l'augmentation index nei soggetti normotesi, suggerendo il ruolo primario degli elevati valori pressori nello sviluppo di queste alterazioni vascolari precoci.

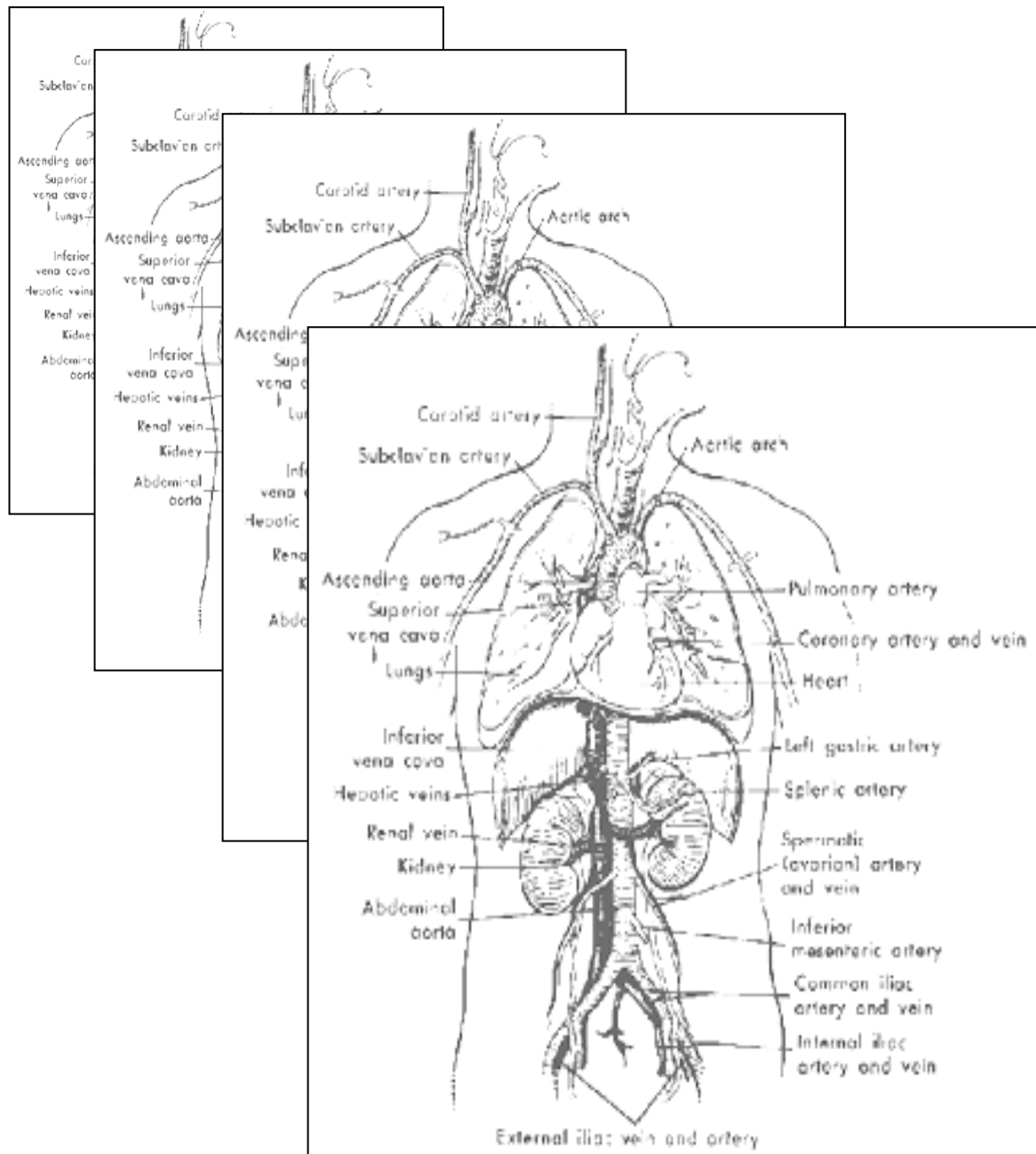
Il livello di attività fisica abituale si associava inversamente con i valori di augmentation index nei soggetti normotesi e in maniera minore nei pazienti ipertesi. Pertanto,

l'ipertensione arteriosa non trattata sembrerebbe ridurre almeno parzialmente l'effetto benefico dell'attività fisica sulla riflessione dell'onda sfignica.

I soggetti anziani che avevano eseguito allenamento regolare, mostravano una migliore vasodilatazione endoteliale dell'arteria omerale rispetto a soggetti sedentari della stessa età. Tale risultato si accompagnava a livelli più elevati di capacità antiossidante plasmatica, suggerendo che una intensa e regolare attività fisica possa prevenire lo stress ossidativo a livello vascolare.

Sebbene l'analisi trasversale della nostra popolazione non abbia mostrato alcuna relazione tra livelli di vitamine antiossidanti C ed E assunte abitualmente con la dieta, l'augmentation index e la funzione endoteliale sia negli ipertesi che nei normotesi, lo studio di intervento ha dimostrato che la supplementazione combinata di vitamina C ed E è in grado di migliorare la vasodilatazione endotelio-dipendente e la rigidità arteriosa in un gruppo di pazienti ipertesi essenziali non trattati. Tale miglioramento era correlato con la riduzione dei markers plasmatici di stress ossidativo. I risultati di questo studio sottolineano la necessità dell'uso di dosi adeguate di differenti antiossidanti per ottenere un effetto benefico sui parametri vascolari.

Infine, i risultati ottenuti nel gruppo di pazienti ipertesi e dei soggetti di controllo suggeriscono che la valutazione della funzione endoteliale nelle arterie periferiche e della rigidità arteriosa, sia centrale che periferica, fornisce misure differenti della funzione e della struttura vascolare. Pertanto nella valutazione della funzione vascolare nell'ottica di una migliore stratificazione del rischio cardiovascolare sarebbe auspicabile in futuro di valutare sia la funzione endoteliale che la rigidità arteriosa.



Acknowledgements

When I left Holland for Italy to do my PhD, I left behind my parents, my sister and my friends. This was the hardest part of my adventure, because all materials and goods are replaceable but family and friends definitively not. However I must say that maybe physically I have felt alone, but mentally hardly ever. This PhD thesis is the fruit of all my work done in the hospital of Pisa, but it is also the fruit of all education, help and love I received from my parents, sister and also my friends. Therefore I would like to thank from the bottom of my heart my father, Johan, my mother, Hieke and my sister Monique for everything that they have done to help me and for everything that they mean to me.

Furthermore, I would like to thank Massimo, for his love, his everlasting patience, stimulation and encouragement and for all his help with the lay out of this thesis. I don't know what I should have done without you.

Of course I want to thank my professor Stefano Taddei, who gave me the chance to perform my PhD in Italy and who told me that after 4 years I still don't understand how it 'works' in Italy, but who also told me that I can always come back if I want to.

Many thanks for professor Antonio Salvetti, who was always available to review my work.

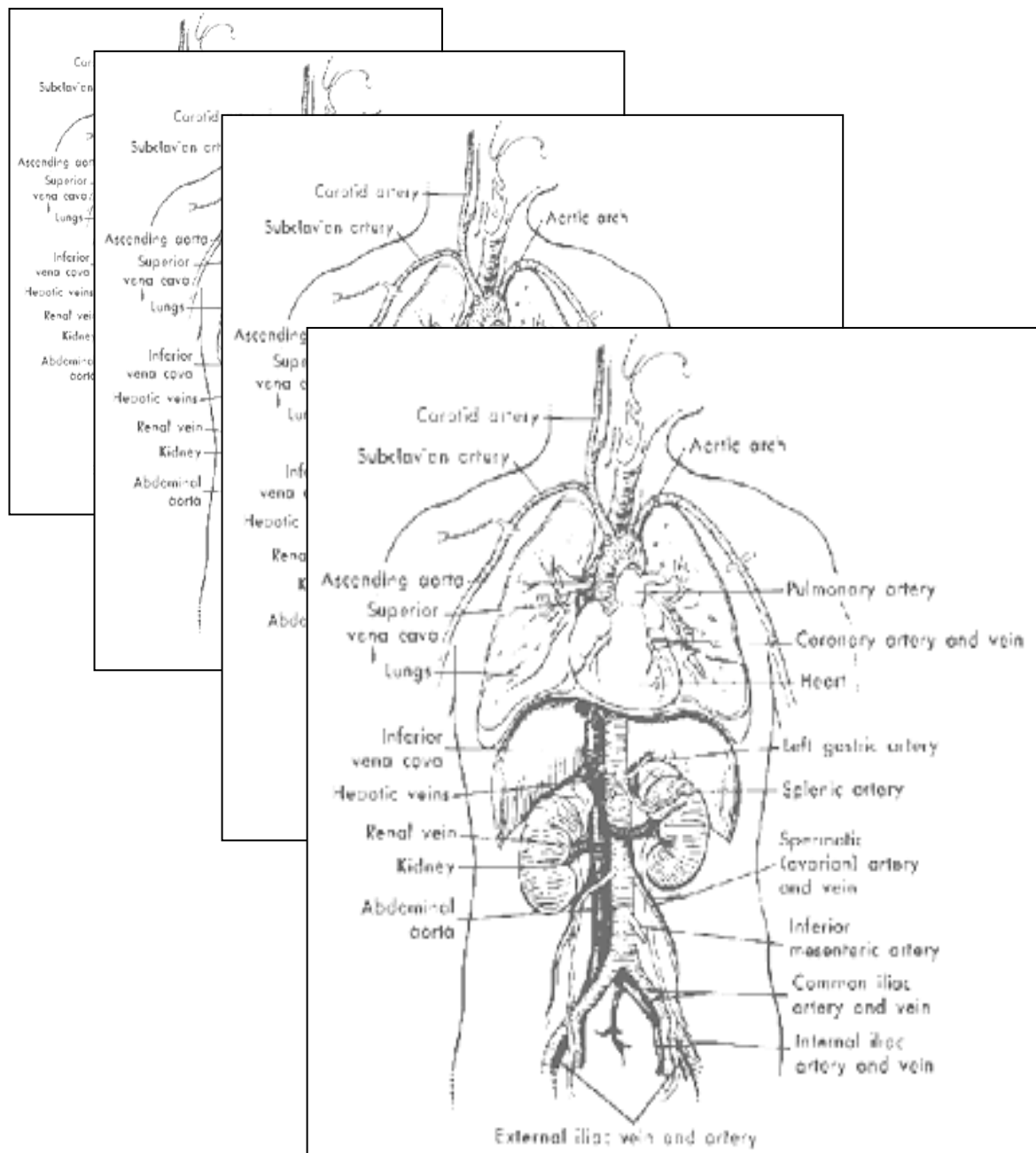
A special thanks for Lorenzo Ghiadoni, my 'capo' or at least he thinks he is. I had a wonderful time working with you.

A lot of thanks also for Dr. Armando Magagna, who recruited almost my entire study population and Antonella who did all the blood sampling. You are not just good colleagues, but also very good friends.

Many thanks to Piero, Stefania and Renzo from the laboratory, who had to deal with a lot of work because of me.

I want to thank Silvija, who came to Pisa from Vilnius, to improve the technique of FMD, but instead of that became one of my best friends and helped me with performing studies and a lot of other things. I would also like to thank Eduardas who codified almost half of the EPIC questionnaires. I hope he stopped having nightmares about it.

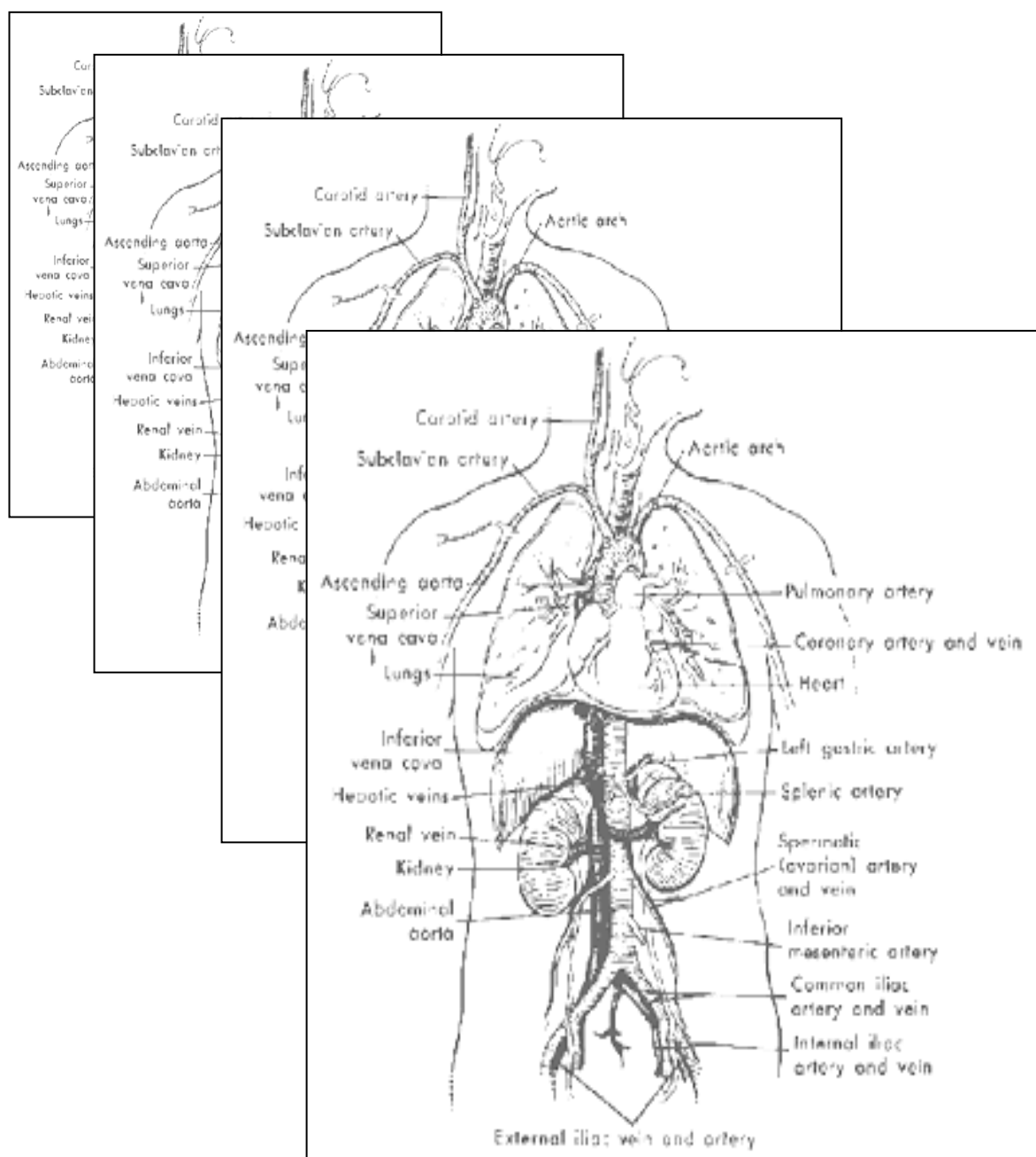
Finally, I would like to thank Domenico Palli, Giovanna Masala, Simonetta Salvini and Marco Ceroti of the CSPO, Scientific Institute of Tuscany for the wonderful collaboration and all the help they gave me with data analysis and the EPIC questionnaires.



List of abbreviations

HT:	hypertensive
NT:	normotensive
FMD:	flow mediated dilation
GTN:	response to sublingual nitroglycerin
RH:	reactive hyperemia
CFPWV:	carotid femoral pulse wave velocity
BPWV:	brachial pulse wave velocity
PPWV:	peripheral pulse wave velocity
AP:	augmented pressure
AIx:	augmentation index
CPP:	central pulse pressure
PP:	pulse pressure
Tr:	time to reflection
ABPM:	ambulatory blood pressure monitoring
DBP:	diastolic blood pressure
SBP:	systolic blood pressure
MAP:	mean arterial pressure
BAD:	brachial artery diameter
CVD:	cardiovascular disease
NO:	nitric oxide
MS:	metabolic syndrome

WC:	waist circumference
BMI:	body mass index
HDL:	high density lipoprotein
LDL:	low density lipoprotein
VO ₂ max:	maximal oxygen uptake
TOSC:	Total Oxyradical Scavenging Capacity
MDA:	malondialdehyde
LOOH:	plasma lipoperoxides
FRAP:	ferric-reducing antioxidant power



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Franzoni F, Plantinga Y, Femia FR, Bartolomucci F, Gaudio C, Regoli F, Carpi A, Santoro G, Galetta F. *Plasma antioxidant activity and cutaneous microvascular endothelial function in athletes and sedentary controls*. Biomed Pharmacother. 2004 Oct;58(8):432-6.

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F. Galetta, F. Franzoni, Y. Plantinga, L. Ghiadoni, M. Rossi, F. Prattichizzo, A. Carpi, S. Taddei and G. Santoro. *Ambulatory blood pressure monitoring and endothelium-dependent vasodilation in the elderly athletes.* Biomed Pharmacother 2006;60(8):443-7.

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Y. Plantinga, L. Ghiadoni, A. Magagna, G. Salvetti, S. Taddei, A. Salvetti. *No differences in augmentation index or endothelial function in essential hypertensive patients with or without the metabolic syndrome.* Subm.

Yvonne Plantinga, Lorenzo Ghiadoni, Armando Magagna, Domenico Palli, Giovanna Masala, Simonetta Salvini, Marco Ceroti, Stefano Taddei, Antonio Salvetti. *Regular intake of antioxidant vitamins, augmentation index and endothelial function in normotensive subjects and essential hypertensive patients.* Subm.

Abstracts

Lorenzo Ghiadoni, Daniele Versari, Agostino Virdis, Alessandro Cipriano, Yvonne Plantinga, Stefano Taddei, Antonio Salvetti. Department of Internal Medicine, University of Pisa, Italy. *Relationship between cardiovascular risk profile and endothelial dysfunction in the forearm microcirculation.* American Society of Hypertension Meeting (ASH), New York, May 2004

Plantinga Y, Ghiadoni L, Galetta F, Franzoni F, Salvetti G, Taddei S, Santoro G, Salvetti A. Department of Internal Medicine, University of Pisa, Italy. *Regular physical exercise prevents age-related decline in plasma antioxidant capacity and endothelium-dependent vasodilation of conduit artery.* European Society of Hypertension (ESH) Meeting, Paris, June 2004

Yvonne Plantinga, Lorenzo Ghiadoni, Melania Bernardini, Ferdinando Franzoni, Fabio Galetta, Stefano Taddei, Antonio Salvetti. Department of Internal Medicine, University of Pisa, Italy. *Habitual physical activity and arterial stiffness in normotensive and hypertensive subjects.* American Society of Hypertension Meeting, San Francisco, May 2005. Presented in a moderated oral session. / Artery 5, Paris 2005. Oral presentation. / European Society of Hypertension 2006, Madrid, poster presentation.

Yvonne Plantinga, Lorenzo Ghiadoni, Armando Magagna, Melania Bernardini, Stefano Taddei, Antonio Salvetti. Department of Internal Medicine, University of Pisa, Italy. *Habitual physical activity, arterial stiffness and endothelial function in normotensive and hypertensive subjects.* European Society of Hypertension Meeting, Milan, June 2005. Presented as poster.

Lorenzo Ghiadoni, Armando Magagna, Daniele Versari, Isabella Kardasz, Yvonne Plantinga, Agostino Virdis, Stefano Taddei, Antonio Salvetti. Department of Internal Medicine, University of Pisa. *Ramipril dose-dependently increases nitric oxide availability in the radial artery of essential hypertensive patients.* American Society of Hypertension Meeting, San Francisco, May 2005 and European Society of Hypertension Meeting, Milan, June 2005. Poster presentation.

E Bianchini, R Andrei, F Faita, V Gemignani, Y Plantinga, M Demi. *Online Measurement of Vasodilation of Peripheral Arteries from Ultrasound Images.* Computers in Cardiology, Lyon, September 2005. Poster presentation.

Plantinga Y, Ghiadoni L, Magagna A, Bernardini M, Taddei S, Salvetti A. Department of Internal Medicine, University of Pisa, Italy. *Supplementation with ascorbic acid and vitamin E improves arterial stiffness and endothelial dependent vasodilation in essential hypertensive patients.* Artery 5, Association for Research into Arterial Structure and

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Ghiadoni L, Plantinga Y, Magagna A, Taddei S, Salvetti A. Department of Internal Medicine, University of Pisa, Italy. *Relationship between measurements of pulse wave analysis and flow mediated dilation in normotensive and hypertensive subjects.* European Society of Hypertension 2006, Madrid, poster presentation.

G.Bernini, E. Benelli, L. Ghiadoni, D. Giannini, Y. Plantinga, C. Taurino, M Bardini, A. Burelli, E. Pucci, A. Pinchera, A. Salvetti. Department of Internal Medicine, University of Pisa, Italy. *Arterial stiffness, endothelial function and oxidative stress in young patients with polycystic ovary syndrome.* European Society of Hypertension 2006, Madrid, poster presentation.